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*MICROINCINERATION STUDIES. III. SHRINKAGE PHENOMENA DURING CARBONIZATION AND ASHING OF WOOD**

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A complete elucidation of the structural complexity of the plant cell wall can result only from the combined application of numerous and diverse methods of investigation. Having found the microincineration technique of value in determining the disposition of intracellular mineral constituents,[1] it seemed desirable to see if it would yield additional information regarding wall structure also. In particular, evidence as to the mineral content of the various wall layers was sought and our earlier findings[2] seemed to show that the so-called middle lamella, particularly the primary walls, contained most of the ash whereas the thick secondary walls were relatively devoid of mineral deposits. It was pointed out that shrinkage of the thin wood sections (5μ) which must be employed in such studies constituted a major technical problem in applying microincineration to an examination of cell wall structure. To counteract such distorting forces strong adhesives must be applied to attach the sections to the microscope slides and with no entirely ash-free adhesive available, the residue from the adhesive itself may be sufficient to compromise the interpretation of the ash picture. On the other hand, where too little adhesive is employed and the sections do not adhere firmly to the slide, a given wall layer might shrink against adjacent layers to render impossible any attempt to indicate distinctions in the amount of inorganic elements present among the various parts. It appeared essential, therefore, to resort to direct observation of the sequence of events involving carbonization and culminating in complete incineration of the thin wood sections in order to evaluate more definitely the significance of results previously obtained.

To this end a special hot stage microscope was devised. The stage consisted of a 25 × 50 mm. aluminum slide chamber, wound with resistance wire, in which temperatures up to 650°C. were attainable. Temperatures were indicated by means of a chromel-alumel thermocouple and a controlling pyrometer of the potentiometer type. A simple doublet lens

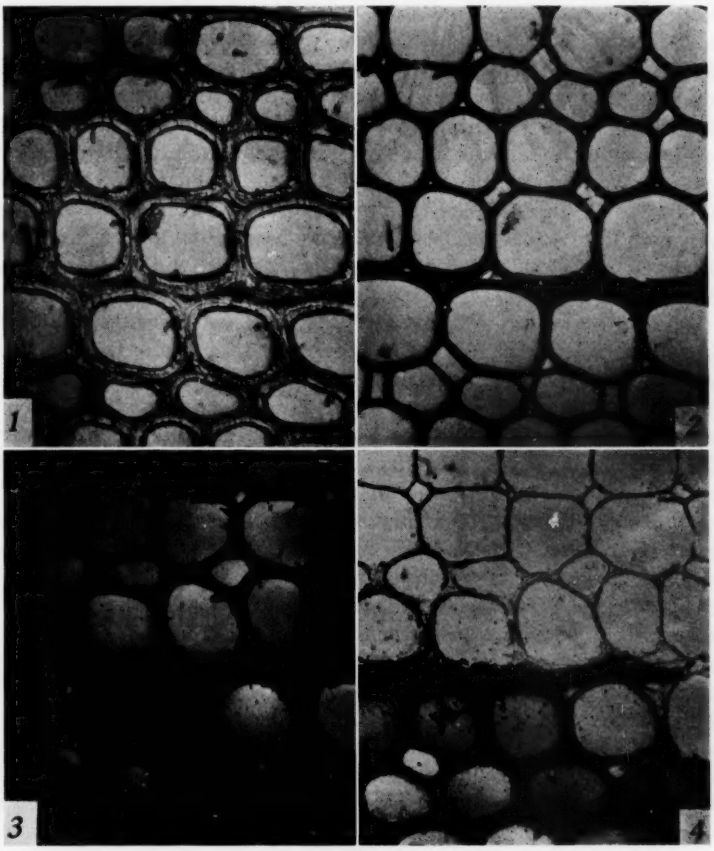
constituted the microscope objective and formed a window for the miniature oven. Useful magnifications of ×100 or ×200 were attained, according to the ocular employed. For photomicrography, the slides were transferred from the hot stage to a microscope with a 4 mm. objective so that magnifications of ×450 could be obtained with a Leitz "Makam" camera.

The wood sections, cut fresh at 5 microns, were attached to microscope slides with a 5 per cent solution of Eastman deashed gelatin, which was then hardened in formaldehyde vapor. The process of incineration in transverse sections of xylem of *Taxodium* sp. and in the thick walled pith cells of young redwood shoots (*Sequoia sempervirens*) was observed under the hot stage microscope. In what follows this process will be briefly described with comments upon the significance of the observations for interpretation of the microincineration results previously reported[2] and for its bearing on the problem of charcoal identification and charcoal structure.

Before proceeding to a description of the shrinkage phenomena observed it will be convenient to distinguish the two general types of distortion involved. The one is characterized by a spatial readjustment whereby the normal disposition of a section, or of an individual cell, is altered either in size or shape. The second type is marked by a reduction in thickness of cell walls only, while the normal topography of the section or cell is unaltered. During the ordinary conversion of wood into charcoal, both types of shrinkage always occur. It is only in the carbonization of thin sections that the latter type alone can be studied, because the strong bond formed between glass and the middle lamella by the gelatin adhesive preserves *in situ* the original boundaries of the cells. Photographic illustrations of the excellent state of topographical preservation obtainable in this manner following complete incineration appeared in the article already cited.[2]

Sections of *Taxodium* observed on the hot stage between 100–200°C. exhibited practically no evidence of distortion. The water lost in this temperature interval corresponds to that evaporated in the oven-drying of lumber commercially where the resultant shrinkage factors for bald cypress are roughly only 6.0 and 3.8 per cent in the tangential and radial directions, respectively. For thin sections attached to glass slides, this relatively small amount of shrinkage during drying would not be readily apprehended visually. It is to be noted, however, that cell walls torn in sectioning or adjacent to pits (cf. Figs. 1 and 2, upper left corner) did contract away from the slide or suffered distortion in various forms, but usually after well up in the temperature range 100–200°C. On several occasions, a semicircular area of a secondary wall was observed breaking away from the primary wall and becoming stretched straight and taut

across the cell lumen. Onset of carbonization as indicated by visible darkening of the walls first became apparent above 200°C. The so-called middle lamella appeared black before the secondary wall, but this may have been owing to its denser structure or its particular refractive proper-



FIGURES 1–4

Transverse sections, 5μ, of xylem of *Taxodium* sp. ×450.

Figures 1 and 2 (*Above*). Identical sections before and after carbonization at 300°C., indicating lateral shrinkage of secondary cell walls.

Figures 3 and 4 (*Below*). Identical sections; figure 3 showing variations in degree of shrinkage at 300°C. as a function of the amount of adhesive employed and figure 4 illustrating how the secondary walls may shrink back toward the middle lamella upon further heating. See text for discussion.

ties rather than to a lower carbonizing temperature. Up to 240°C., at which temperature marked carbonization had already occurred, the shrinkage forces had not yet played a dominant part. Even in the case of sections lying comparatively free beneath a coverslip (no adhesive) and subjected to temperatures as high as 265°C., only minor contractions might result. But above 275°C., secondary walls began to show marked shrinkage and were held in place with difficulty so that a generous application of adhesive was essential. Oftentimes the sections split along, and contracted away from, the medullary rays with the consequent deformation of neighboring cells. At 300°C., the walls, black and opaque, may still have undergone no obvious shrinkage, but usually an exposure of several hours at this temperature resulted in a lateral reduction of the broad secondary walls to $^1/_2$ or $^1/_3$ of their former width. Such a transformation is depicted in figures 1 and 2, which show the same transverse section of *Taxodium* before and after carbonization at 300°C. Superposition of these two photomicrographs revealed no reduction in the dimensions of the section as a whole, but a marked decrease in the width of the individual cell walls by a factor of $1^1/_2$ to 2. The intercellular spaces following carbonization are very much enlarged and decidedly conspicuous, for their contours of inverted curves can offer little mechanical resistance to contractile forces, with the result that these curved segments tend to straighten. At the same time, a slight angularity is imparted to the outline of the lumen.

It will have been evident from earlier remarks that the rôle assumed by the adhesive in this study is an important one. A particularly good illustration of this fact is furnished in figure 3, which shows the transitional character of the carbon picture between regions of varying gelatin concentration. Below, where the gelatin smear was heaviest as evidenced by its carbon residue in the lumens, little or no shrinkage in wall width took place; in the region above and to the right, which received least gelatin, the walls are about $^1/_2$ of their natural width. The sections photographed in figures 2 and 3 underwent the same heat treatment, being held above 250°C. for $1^1/_2$ hours while attaining a maximum of 300°C., and it is clear that the decrease in wall width in the upper portion of figure 3 is approximately the same as that characteristic of the entire section shown in figure 2. At still higher temperatures, the process of carbonization proceeded more and more vigorously so that in the range 325–340°C. there was violent lateral contraction of the secondary walls. As a result, the section in figure 3 has been converted into that of figure 4. Here again, we have a photomicrograph of a transition stage, and a very important one, for it reveals the steps in a process by which the broad secondary wall may shrink back into a narrow semi-cylindrical mass on either side of the middle lamella, the degree of shrinkage depending upon the amount of adhesive employed. It will be seen that in figure 4 oxidation

was well under way and ash residues already conspicuous. The double nature of the wall residue, evidenced in the upper portion of figure 4, was interpreted in the first paper of this series[2] as denoting an absence of ash in the intercellular substance. Now that the origin of these deposits on either side of the intercellular layer has been explained, it is still to be noted that whatever ash may be present in the intercellular material is rendered inconspicuous by the combined residues of the other wall layers. As already stated, our earlier microincineration evidence indicated almost complete absence of inorganic elements in the secondary walls of *Taxodium*. On the basis of the information just given this interpretation is subject to question. Thus, in figure 4, where the walls were able, because of lack of sufficient adhesive, to contract into narrow bands, no evidence as to the mineral content of the secondary wall can be obtained. Where the amount of adhesive was greater, and shrinkage inhibited, ash is present throughout the wall but here it is largely, or perhaps entirely, the residue of the gelatin adhesive as indicated by the deposits in adjacent cell lumens. In both cases it is indeterminate as to how much ash, if any, came from the secondary walls and it seems evident that the problem can be solved by the microincineration method only after an appropriate ash-free adhesive is available.

Since the technical difficulties encountered in the case of *Taxodium* were so great, efforts were made to secure more favorable material from which critical evidence might be obtained concerning the ash content of the secondary wall. At the suggestion of Dr. I. W. Bailey, who also was kind enough to supply the sections, thick walled pith cells of redwood (*Sequoia sempervirens*) were observed during the process of incineration. This material proved more desirable than *Taxodium* in that the pith cells are larger and have less tendency to shrink during heating. In several instances, when sections were confined beneath a coverslip without adhesive, segments of walls were found still intact following incineration. These contained diffuse ash residues in the secondary layer. This finding was amply confirmed in sections where gelatin was employed in amounts so slight as to leave but a negligible, if at all detectable, residue in the cell lumens. The presence of ash in secondary walls of redwood pith cells is significant and leads one to expect inorganic residues in secondary walls of other tissues and species. However, there exists the possibility that these cells may be specialized with respect to ash content, although it seems improbable that the pith would be unique in this regard. It is also to be noted that redwood pith cells always left an abundant ash along the internal boundaries of both lumens and intercellular spaces.

Evidence derived from the carbonization of thin sections can be brought to bear on a number of charcoal problems, both theoretical and practical. With regard to the histological analysis of charcoal and charred woods, for

example, it has demonstrated how important it is to take into account the factor of shrinkage and how hazardous to base identifications on cell size and shape relationships alone (cf. also Maby[3]). Tanner,[4] who was interested in tracing the origin of decolorizing charcoals, proposed a system of identification based on the form, size and arrangement of the perforations observable in microscopic fragments. These minute perforations were ascribed to pit apertures, hence the so-called "identification particles" were regarded as longitudinal fragments of cell walls. On the basis of the extreme shrinkage observed during carbonization of transverse sections, it is entirely possible that cell lumens might be so reduced in size in some species as to simulate pit apertures. Indeed, at least one of the twelve photomicrographs published by Tanner (poplar) appears to be a transverse section. The insufficiency of determinations made solely on such unoriented microscopic fragments is apparent. In stressing the lack of reliable diagnostic criteria for the positive identification of woods even where large oriented sections are available, Bailey and Faull[5] have pointed out that "such supposedly conservative qualitative characters as form and orientation of pits, or of pit apertures, fluctuate considerably not only in trees grown under markedly different environmental conditions but also within different parts of a single tree."

Density and porosity data for commercial wood charcoals indicate shrinkage values approximately the same as those found in the carbonization of thin sections described here. Yields of charcoal from moisture-free wood average about 30 per cent by weight[6] while the real densities of wood cellulose and charcoal are about 1.57[7] and 1.50,[8] respectively. Since the density of the solid substance has remained practically constant during a weight loss of 70 per cent, a corresponding decrease in actual volume would be anticipated. Nearly half of this shrinkage can be accounted for by the reduction in external dimensions; the remainder is internal shrinkage and results in the marked increase of porosity from 65 per cent in the original wood to 82 per cent in the derived charcoal, for a given sample. Under the restraining forces present in intact wood, the increase in porosity probably results from volume changes in intercellular spaces and pit areas, and from the creation of minute cavities within the walls themselves rather than from greatly enlarged cell lumens. Microscopical observations on the carbonization of cut sections indicate that a denser charcoal would be obtained from pulverized wood, where the forces opposing shrinkage are greatly diminished, than from coarser samples. Experimental evidence in harmony with this view has been reported by Pickles,[9] who found birch charcoal from chips and dust to have real densities of 1.44 and 1.47 grams/cm.^{3}, respectively. Likewise Cude and Hulett[10] found a greater density for smaller sized particles in the case of cocoanut charcoal.

Any attempt to interpret precisely the shrinkage phenomena occurring in wood during carbonization on the basis of the structural rearrangements in the cellulose molecule would be highly speculative. X-ray diffraction studies by Debye and Scherrer[11] have indicated a structure for charcoal similar to that of graphite, but consisting of numerous small crystalline micellæ. Since little is known, not only of the forces existing between the micellæ but also of the lateral linkage bonds between cellulose molecules, it is obviously impossible to predict the extent of spatial readjustment incident upon carbonization.

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IRIDOPHYCUS GEN. NOV. AND ITS REPRESENTATION IN SOUTH AMERICA

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Iridaea Bory (Diction. class. d'hist. nat., 9: 15, 16, Feb. 1826) is antedated by the genus *Iridea* Stackhouse (Ner. Brit., ed. alt., p. IX, 1819), a synonym of *Desmarestia* Lamouroux (Essai, 25, 1813). Bory's genus was of ill assorted composition and at least two very different species have been established as types: *Fucus edulis* Stackh. (also type of *Dilsea* Stackh., 1809) by Greville in 1830 (Algae Britt., 157) and *Fucus cordatus* Turner by J. G. Agardh in 1876 (Spec. Alg., 3 (1), 179) who finally delimited and defined *Iridaea* as usually accepted, on the basis of the latter species. It seems best, therefore, to coin a new name for the genus, rather than to suggest the retention of *Iridaea* as a *nomen conservandum*. We propose, therefore, the name *Iridophycus* to include those species belonging

to the genus as limited by J. G. Agardh in 1876, with the designated type species *Iridaea capensis* J. Ag. (K. V. Akad. Handl., 1847, p. 85, pl. 1), since the *Fucus cordatus* Turn., owing to the seeming absence of a type specimen, is as yet somewhat confused as to exact identity. The type of our proposed new genus is, therefore, to be designated as *Iridophycus capensis* (J. Ag.) comb. nov. (*Iridaea capensis* J. G. Agardh, K. V. Akad. Handl., 1847, 85, pl. 1). The genus is limited to those members of the Gigartinaceae whose medulla is made up of reticulate filaments, with both cystocarps and tetrasporangial sori immersed in the frond, with the cystocarps provided with a *pericarpium proprium*, and with the tetrasporangia accessory in origin.

The South American species are in much confusion, not only as to specific limits, but also as to nomenclature, because of the changes of names proposed by Bory de Saint Vincent in his three separate publications, resulting in a synonymy especially difficult to unravel since, from our present point of view, at least, we cannot accept Bory's final opinion as to the reduction of some of his earlier proposals to synonymy.

The South American species may be listed as follows:

I. EUIRIDAEA J. G. Agardh, Spec. Alg. 3 (1): 180, 1876 (lim. mut.). Apophysis smooth on both margins and surface.

1. *Iridophycus Boryanum* sp. nov.
   Iridaea laminarioides Bory, p.p., Voy. Coq., 105, (pl. juniores, pl. 11, figures 1, *A*, *B*, *C*), 1827; *I. heterococca* Kuetzing, Tab. Phyc., 17: 4, pl. 11, 1867 (parasitized conditions!).
2. *Iridophycus obovatum* (Kuetz.) comb. nov.
   Iridaea obovata Kuetzing, Spec. Alg., 728, 1849; *I. micans β obovata*, Kuetzing, Tab. Phyc., 17: 3, pl. 9, 1867.
3. *Iridophycus micrococcum* (Kuetz.) comb. nov.
   Iridaea micrococca Kuetzing, Tab. Phyc., 17: 4, pl. 12, figures *a*, *b*, 1867.
4. *Iridophycus laminarioides* (Bory, p.p.) comb. nov.
   Iridaea laminarioides Bory, Voy. Coq., 105, (pl. adult., pl. 11, figure 1, *D*, *E*), 1827.
5. *Iridophycus micans* (Bory) comb. nov.
   Iridaea micans Bory, *in* D'Urville, Fl. des Malouines, 594, 1826; Voy. Coq., 110, pls. 13, 13 bis, 1827.
6. *Iridophycus caespitipes* sp. nov.
   Thallus caespitosus, usque ad 7–20 (?) cm. altus, e expanso horizontaliter lato progressive oriendus, corneo-cartilagineus, apophysi elongata, usque ad 1–1.5 cm. alto, anguste, 1 mm., usque ad 2 mm. diam., sub laminam amplificante, exsiccata canaliculata, in laminam cuneatum usque ad subcordatum,

marginibus superficiebusque levibus, abruptissime expansa; laminis e cuneato-lanceolatis, usque ad late subcordato-reniformibus, superficiebus levibus, marginibus lente repandis, integris; cystocarpiis in lamina dense sparsis; antheridiis nondum visis; soris tetrasporangiiferis in medio laminarum immersis, accessore oriendis, exsiccatis superficie utraque lente tumescentibus.

Spec. typ.: cystocarpic, Magdalen Islands, Straits of Magellan, Hassler Exp. (No. 2066, Herb. William Randolph Taylor); tetrasporic (imperfect), Falkland Islands, Mrs. Elinor Vallentin (Herb. Kew. and Herb. Univ. Calif., No. 207,076).

II. PORPHYRIDAEA J. G. Agardh, Spec. Alg., 3 (1): 181, 1876.
Apophysis elongated, flat, thin, simple or sparingly dichotomous, irregularly foliose-proliferous from both margins and surfaces.

7. *Iridophycus membranaceum* (J. Ag.) comb. nov.
Iridaea membranacea J. Ag., Lunds Univ. Årsskr., 8 : 8, 1872: K. Sv. V. Akad. Handl., 15 (6): pl. 10, 1879.

III. CHONDRIRIDAEA subgen. nov. Apophysibus repetite et moderate regulariter dichotomis, fimbriis foliolisque plus minusve adornatis.

8. *Iridophycus dichotomum* (Hook. f. et Harv.) comb. nov.
Iridaea dichotoma Hooker f. et Harv., Lond. Jour. Bot., 4: 262, 1845; Kuetzing, Tab. Phyc., 17: 5, pl. 13, figures *c*, *d*, 1867. *I. dentata* Kuetzing, Spec. Alg., 728, 1849; Tab. Phyc., 17: pl. 14, 1867.

IV. GIGARTIRIDAEA subgen. nov. Apophysibus simplicibus aut rare semel furcatis, marginibus et vulgo superficiebus fimbriis gracilibus robustisve adornatis.

9. *Iridophycus undulosum* (Bory) comb. nov.
Iridaea undulosa Bory, in D'Urville, Flore des Malouines, 594, 1826 (non *I. Augustinae* Bory, Voy. Coq., 109, 1827, plantis junioribus sicut pl. 12, fig. E, exceptis).

10. *Iridophycus ciliatum* (Kuetz.) comb. nov.
Iridaea ciliata Kuetzing, Spec. Alg., 726, 1849; Tab. Phyc., 17: 4, pl. 10, 1867.

11. *Iridophycus crispatum* (Bory) comb. nov.
Iridaea crispata Bory, Dict. class. d'hist. nat., 9: 16, 1826; *I. Augustinae* Bory, Voy. Coq., 108, 1827 (p.p., maj., sicut pl. 12, figures *A-D*).

It seems desirable to present here a brief, but tentative, key to the South American species of *Iridophycus:*

1. Apophysis with margins and surfaces smooth (EUIRIDAEA J. Ag.) 2
1. Apophysis with outgrowths on margins and (usually) on surfaces.. 7
2. Base of blade never truly cordate..... 3
2. Base of blade truly cordate (when well developed)..... 6
3. Margins of blade short-ciliate.....4. *I. laminarioides* (verum)
3. Margins of blade not ciliate..... 4
4. Apophysis broad and elongated, canaliculate cucullate.....
.....1. *I. Boryanum*
4. Apophysis short, narrow, flat..... 5
5. Blades thin, membranaceo-chartaceous.....3. *I. micrococcum*
5. Blades thick, carnose-cartilaginous.....2. *I. obovatum*
6. Fronds arising singly (or very few) from a basal disc...5. *I. micans*
6. Fronds, numerous, arising from a prostrate expanding basal thallus.
.....6. *I. caespitipes*
7. Apophysis very much elongated, flat, thin, foliose-proliferous from margins and surfaces (II. PORPHYRIDAEA J. Ag.).....
.....7. *I. membranaceum*
7. Apophysis with fimbriae, spines or warts from margins (and usually from surfaces)..... 8
8. Apophysis several times dichotomous, with fimbriate margins (III. CHONDRIRIDAEA S. et G.).....8. *I. dichotomum*
8. Apophysis simple (IV. GIGARTIRIDAEA S. et G.)..... 9
9. Apophysis elongated, cuneate, canaliculate; base of blade truly cordate; outgrowths slender, acute.....11. *I. crispatum*
9. Base of blade cuneiform to reniform; outgrowths not acute..... 10
10. Apophysis short, broad; outgrowths slender, simple.....
.....9. *I. undulosum*
10. Apophysis short, broad; outgrowths stout, low, usually compound..
.....10. *I. ciliatum*

Iridaeis alteris maxime austro-americanis adhuc non rite cognitis.

1. *Rhodoglossum macrodontum* (Skottsb.) comb. nov.
Iridaea macrodonta Skottsberg, K. Sv. V. Akad. Handl., 63 (8): 8, 9, figure 1 *b*, 1923.
2. *Rhodoglossum pulchrum* (Kuetz.) comb. nov.
Iridaea pulchra Kuetzing, Spec. Alg., 725, 1849, Tab. Phyc., 17: 2, pl. 5, figures *c*, *d*, 1867.
3. *Gigartina papillosa* (Bory) comb. nov.
Iridaea papillosa Bory, *in* Dict. class. d'hist. nat., 9: 6, 1826; *I. radula* Bory, Voy. Coq., 107, 1827 (p.p.).
4. *Gigartina Skottsbergii* sp. nov.
Iridaea radula Hook. f. et Harv., Flora Antarctica, II: 485, 1847

(p.p.maj.) et Auctorum ut plantis austroamericanis sub *I. radulam* referentibus, incl. Skottsberg, K. Sv. V. Akad., Handl., 63 (8): 9, 1921 (p.p.).

Thallus prostratus, dorsiventralis, primo vulgo orbicularis, mox reniformis, initio umbilicatus per discum eccentricam superficiei inferae, sed mox per hapteres numerosos secundarios concentricos circum discum primordialem oriendos affixus, plus minusve crassus, laete vinoso-ruber, aetate provecta, irregulariter et late lobatus, marginibus repandis, nunc dentatis, nunc papillosis, nunc levibus; papillis curtis robustisque, in superficiem superam laxe usque ad dense, in superficiem inferam paucis aut nullis, vestitus, zona intramarginali angusta, nuda: soris in substantiis laminae immersis, in frondibus exigue papillosis positis, tetrasporangiis accessore oriendis; cystocarpiis fere globulis curte robusteque pedicellatis et apiculatis, superficie una alteraque, maxime supera, dense sparsis, interdum quoque marginalibus, pericarpium proprium crassum ostendentibus; antheridiis superficiem superam fere omnem obducentibus.

Spec. typ.:—tidepools, Slogget Bay, Fuegia, cystocarpic and antheridial (Herb. Univ. Calif., No. 205,704), Carl Skottsberg; tetrasporic, Falkland Islands (Waldo L. Schmitt, No. 113, in Herb. Wm. Randolph Taylor and fragment of same in Herb. Univ. Calif., No. 472,534).

From Falkland Islands to Cape Horn and in Chile (Valparaiso).

5. *Phyllymenia Belangerii* (Bory) comb. nov.

   Iridaea Belangerii Bory, *in* Belanger, Voy. Aux Ind., orient., etc., Bot. part 2: 160, pl. 15, fig. 1, 1846!; *P. hieroglyphica* J. G. Agardh, K. V. Akad., Handl., 1847: 86, pl. 2 (non *Iridaea capensis* J. Ag.!!).

6. *Phyllymenia cornea* (Kuetz.) comb. nov.

   Iridaea cornea Kuetzing, Tab. Phyc., 17: 6, pl. 20, 1867; *Cyrtymenia cornea* Schmitz, Nuova Notarisia, 7: 16, 1896.

7. *Iridaea fissa* Suhr.

   Suhr, Flora, 19 (1): 340, pl. III, figs. 26 and L, 1836; *Gigartina fissa* J. G. Agardh, Spec. Alg., 3 (1): 201, 1876 p.p. (credited to the region of Cape Horn) seems probably a case of confusion of locality with that of the Cape of Good Hope. It is most probably the tetrasporic plant of *Gigartina stiriata* (Turn.) J. Ag., as is also *Iridea clavellosa* Suhr (Flora, 19 (1): 341, pl. III, figures 27, 28, 1836), credited also to Cape Horn. The type specimens (in Herb. Suhr?) have not been examined.

8. *Callymenia pustulosa* (P. & R.) comb. nov.

   Iridaea pustulosa Postels et Ruprecht, Ill. Alg., 18, pl. 32, 40, figures 94, 95, 1840.

LINKAGE RELATIONS OF HYDROCEPHALUS (hy_1) IN THE HOUSE MOUSE, MUS MUSCULUS[1]

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Two recessive types of hereditary hydrocephalus ("water on the brain") have been reported in the house mouse, *Mus musculus*. The first of these, known as the American type (hy_1), arose in a flexed-tail strain of mice at Michigan State College, East Lansing, Michigan, and its inheritance and anatomical basis have elsewhere been described in detail (Clark 1932, 1934). The second hydrocephalus (hy_2), known as the German type, appeared among the descendants of wild caught mice reared at the Kaiser Wilhelm Institute for Brain Research in Berlin, Germany, and it has been studied genetically and anatomically by Dr. Klaus Zimmermann (1933, 1935). These two brain anomalies although phenotypically similar depend upon different recessive genes for their expression (Clark, 1935).

In the present study, the American form of hydrocephalus (hy_1) was tested for linkage with 15 other mutant characters whose genes are located in 14 of the 20 pairs of chromosomes of the house mouse. In table 1 are shown the F_2 linkage data from crosses between hydrocephalus and the following characters: Roberts' pink-eye (p_2), albinism (c), dilution (d), leaden (l), flexed-tail anemia (f), piebald (s), dwarf (dw), Zavadskaïa shaker (sh_2), waltzing (v) and rodless (r). In each case, a simple dihybrid 9:3:3:1 ratio is expected if no linkage exists. The observed ratio, the expected 9:3:3:1 ratio and the deviations divided by their respective probable errors are given for each cross.

In the test with Roberts' pink-eye, there is a decided deficiency of double recessive pink-eye hydro mice, the deviation from the expected number being nearly 4 times its probable error. Since the mice in this class result from the union of crossover gametes such a deficiency would indicate linkage between hydrocephalus and Roberts' pink-eye were it not for the fact that the other three classes fit the expected numbers so closely. Roberts and Guisenberry (1935) have demonstrated that pink-eye (p_2) is linked with agouti. Since I show later that hydrocephalus is not linked with agouti (table 2), we may conclude that it is not linked with Roberts' pink-eye and that the deficiency in the observed pink-eye "hydro" class is due to the increased lethal action of the hydrocephalus gene when combined with the gene for Roberts' pink-eye.

In tests for linkage of hydrocephalus with albinism, blue dilution, leaden, flexed-tail anemia, piebald and waltzing, the observed number

TABLE 1

F_2 DATA FROM LINKAGE TESTS BETWEEN HYDROCEPHALUS (hy_1) AND OTHER MUTANT CHARACTERS OF THE HOUSE MOUSE

The expected 9:3:3:1 ratio and the deviations divided by their respective probable errors are given for each cross. *X* indicates the dominant allelomorph of the character tested, *x* its recessive allelomorph.

		X Hy_1	*x* Hy_1	*X* hy_1	*x* hy_1	TOTAL NO.
Pink-eye (Roberts')	Observed	95	30	35	2	162
	Expected	91.1	30.4	30.4	10.1	
	Dev./P.E.	0.92	0.19	1.37	3.89	
Albinism	Observed	96	21	20	6	143
	Expected	80.5	26.8	26.8	8.9	
	Dev./P.E.	3.89	1.84	2.16	1.49	
Dilution	Observed	67	13	15	6	101
	Expected	56.8	19.0	19.0	6.3	
	Dev./P.E.	3.04	1.51	2.26	0.18	
Leaden	Observed	64	16	21	7	108
	Expected	60.8	20.2	20.2	6.8	
	Dev./P.E.	0.92	1.53	0.29	0.18	
Flexed-tail anemia	Observed	60	12	13	4	89
	Expected	50.1	16.7	16.7	5.5	
	Dev./P.E.	3.16	2.48	2.48	1.54	
Piebald	Observed	70	26	17	5	118
	Expected	66.4	22.1	22.1	7.4	
	Dev./P.E.	0.99	1.36	1.78	1.36	
Dwarf	Observed	71	19	5	5	100
	Expected	56.2	18.8	18.8	6.2	
	Dev./P.E.	4.42	0.82	5.27	0.74	
Shaker (sh_2) (Zavadskaïa)	Observed	149	49	44	6	248
	Expected	139.5	46.5	46.5	15.5	
	Dev./P.E.	1.80	0.60	0.60	3.70	
Waltzing	Observed	42	17	13	3	75
	Expected	42.2	14.1	14.1	4.7	
	Dev./P.E.	0.07	1.27	0.48	1.21	
Rodless	Observed	Discarded	Discarded	23	5	28
	Expected	.....	.....	21.0	7.0	
				1.30	1.30	

in the critical double-recessive class in each case approximates the expected number very closely. The normal classes in the albino and dilute crosses are slightly in excess of expectation but this could easily be due to differential viability. The F_2 data from these crosses indicate that hydrocephalus is not linked with albinism, dilution, leaden, flexed-tail anemia, piebald or waltzing.

In the F_2 linkage test with dwarf the excess of normal mice and the deficiency in the hydro non-dwarf class is probably due to differential viability. The mortality among the F_2 young of this cross was extremely high due to para-typhoid infection. Nearly the expected number of dwarf "hydro" mice was obtained and since they are produced entirely by crossover gametes, it seems highly probable that the genes for hydrocephalus and dwarf lie in different chromosomes.

In the linkage test with rodless retina, mice known to be homozygous for this defect were crossed with hydrocephalus animals. On the basis of free segregation, one would expect in the F_2 a ratio of 9 normal:3 rodless "non-hydro":3 "hydro" non-rodless:1 hydro rodless. Or, considering the "hydros" alone (last two classes) we should expect one "hydro" out of four to be rodless. Since this is a repulsion cross, if these two characters were linked, less than one-fourth of the hydrocephalus mice should be rodless; and the stronger the linkage, the greater this deficiency would be. For this reason, I considered it sufficient to section the eyes of the "hydros" only and discard the "non-hydros." Five out of 28 F_2 hydrocephalus mice proved to be homozygous for rodless retina indicating that the genes for hydrocephalus and rodless have their loci in different chromosomes.

In a recent paper (Clark 1935) dealing with the linkage relations of Zavadskaïa shaker in the house mouse, I indicated that a linkage might exist between that character and hydrocephalus. Since then more reliable results have been obtained and they are listed in table 1. The observed number in the double-recessive shaker-"hydro" class is below expectation, the deviation being 3.7 times its probable error. The other three observed classes, however, fit the expected numbers very closely. If there is not linkage between hydrocephalus and shaker (sh_2), two-thirds of the F_2 shaker non-"hydro" mice should be heterozygous for hydrocephalus. Fourteen shakers from this class were saved and mated with animals heterozygous for hydrocephalus. Ten of the 14 shakers proved to be heterozygous for hydrocephalus and each one of these 10, therefore, must carry one crossover gamete. These tests prove conclusively that "hydro" and Zavadskaïa shaker are independently inherited.

Table 2 contains the back-cross data from linkage tests between hydrocephalus and the following characters: agouti, chocolate, brachyury, dominant spotting (*Ww*) and naked. In each cross the data were obtained

by crossing F_1 doubly heterozygous mice with animals heterozygous for hydrocephalus but homozygous and recessive for the other character. Such a modified backcross gives an F_2 ratio for hydrocephalus and a backcross ratio for the other character. The observed ratio, the expected 3:3:1:1 ratio and the deviations divided by their respective probable errors are given for each cross.

The linkage test between hydrocephalus and agouti is of special significance because of the possible linkage between "hydro" and Roberts'

TABLE 2

BACK-CROSS DATA FROM LINKAGE TESTS BETWEEN HYDROCEPHALUS (hy_1) AND OTHER MUTANT CHARACTERS OF THE HOUSE MOUSE ($Xx\ Hy_1\ hy_1 \times xx\ Hy_1\ hy_1$)

The expected 3:3:1:1 ratio and the deviations divided by their respective probable errors are given for each cross. X indicates the dominant allelomorph of the character tested, x its recessive allelomorph.

		$X\ Hy_1$	$x\ Hy_1$	$X\ hy_1$	$x\ hy_1$	TOTAL NO.
Agouti	Observed	46	37	12	11	106
	Expected	39.8	39.8	13.2	13.2	
	Dev./P.E.	1.65	0.75	0.52	0.96	
Chocolate	Observed	14	14	3	4	35
	Expected	13.1	13.1	4.4	4.4	
	Dev./P.E.	0.42	0.42	1.06	0.30	
Brachyury	Observed	48	48	12	18	126
	Expected	47.2	47.2	15.7	15.7	
	Dev./P.E.	0.2	0.2	1.5	0.9	
Dominant spotting (*Ww*)	Observed	21	20	5	4	50
	Expected	18.8	18.8	6.2	6.2	
	Dev./P.E.	0.85	0.85	0.76	1.39	
Naked	Observed	31	42	8	13	104
	Expected	39.0	39.0	13.0	13.0	
	Dev./P.E.	2.15	0.81	2.19	0.00	

pink-eye indicated by data already discussed in table 1. If "hydro" is linked with Roberts' pink-eye, it should also exhibit linkage with agouti since the genes for pink-eye (p_2) and agouti lie in the same chromosome (Roberts and Guisenberry, 1935). It can be seen, however, from table 2, that the observed ratio in the cross with agouti fits the expected ratio very closely. These results in conjunction with those given in table 1 (Roberts' pink-eye cross) indicate that the hydrocephalus gene does not lie in the chromosome occupied by the genes for agouti and Roberts' pink-eye.

The other data in table 2 demonstrate that hydrocephalus segregates

independently of chocolate, brachyury, dominant spotting and naked (dominant hairless). In every case the observed ratio approximates the expected 3:3:1:1 ratio very closely.

The data in tables 1 and 2, although not so extensive as might be desired, justify the conclusion that hydrocephalus is not linked with any of the 15 characters tested. Since the genes for these 15 characters of the house mouse are, on good evidence, believed to be located in 14 different pairs of chromosomes, it follows that the gene for hydrocephalus must lie in an independent fifteenth chromosome.

Summary.—The hydrocephalus character (hy_1) of the house mouse (*Mus musculus*) has been tested for linkage with 15 other characters whose genes serve as markers for 14 of the 20 chromosomes of the mouse. Since no linkage was observed with any of these characters, it follows that the gene for hydrocephalus (hy_1) must be located in a fifteenth chromosome.

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A FURTHER STUDY OF REGULARITY OF NUTRITIONAL RESPONSE TO CHEMICAL INTAKE

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Rats kept for several generations in the laboratories of Osborne and Mendel have tended to show progressive increase in rapidity of growth and in size and apparent well-being.[1-4] In our own colony, originally derived from the same stock, we have observed that even upon a diet of approximately minimal adequacy in some respects (the Diet A described in previous papers[5-7]) successive generations have tended to show earlier attainment of maturity and a slightly larger average size at all ages; but no im-

provement in the average length of life of either sex, or in the percentage of females rearing young.

Thus in the combined experience of the two laboratories, different strains of the same original stock have become adapted to different diets, and it becomes of scientific interest to determine whether the nutritional responses to enrichment of chemical intake are thereby affected. For inasmuch as only about half of the females of each generation rear young on our Diet A, the families still thriving after eight generations on this diet might be expected to indicate whether or not adaptation or habituation to a particular régime materially influences nutritional responses.

TABLE 1

COMPARISON OF RESULTS ON DIETS A AND B FED TO INDIVIDUALS FROM FAMILIES WHICH HAD THRIVED FOR EIGHT GENERATIONS ON DIET A

	ON DIET A		ON DIET B			
	NO. CASES	AVERAGE	NO. CASES	AVERAGE	DIFFERENCE WITH ITS P. E.	DIFFERENCE ÷ ITS P. E.
Gain in body weight during 5th to 8th week of life, per 1000 calories of food consumed, *gms.*	68	76.6 ± 0.75	74	87.0 ± 0.7	10.4 ± 1.0	10.4
Gain in body weight during 5th to 8th week of life, per gram of protein consumed, *gms.*	68	2.17 ± 0.02	74	2.26 ± 0.02	0.09 ± 0.027	3.3
Age of females at birth of first young, *days*	65	133.5 ± 3.4	84	107.9 ± 1.4	25.6 ± 3.7	6.9
Duration of reproductive life, *days*	67	135.8 ± 7.8	67	211.5 ± 8.8	85.7 ± 11.7	7.6
Number of young borne	67	19.9 ± 1.3	67	29.1 ± 1.2	9.2 ± 1.8	5.1
Number of young reared	67	5.4 ± 0.6	67	9.8 ± 0.7	4.4 ± 0.9	4.9
Average weights of young at 28 days, *gms.*	365	35.3 ± 0.17	658	39.4 ± 0.13	4.1 ± 0.21	20.
Length of life:						
Males, *days*	44	565 ± 9.9	45	623 ± 9.0	58 ± 13.4	4.3
Females, *days*	68	606 ± 8.5	65	671 ± 9.4	65 ± 12.6	5.1
Percentage of females rearing young	67	45	67	77	.....	..

To test this question, young rats from the ninth generation of families which had thrived on Diet A (the "poorer" or "minimal adequate" dietary) were divided into accurately matched lots, as described in our previous papers, one of each pair of such lots being continued upon Diet A while the duplicate lot was fed Diet B, which is of slightly higher protein content than Diet A, and materially richer in calcium, in vitamin A and in vitamin G (lactoflavin or "the flavin factor").

Notwithstanding their eight generations of adaptation to the "minimal adequate" Diet A, these animals showed more efficient growth and develop-

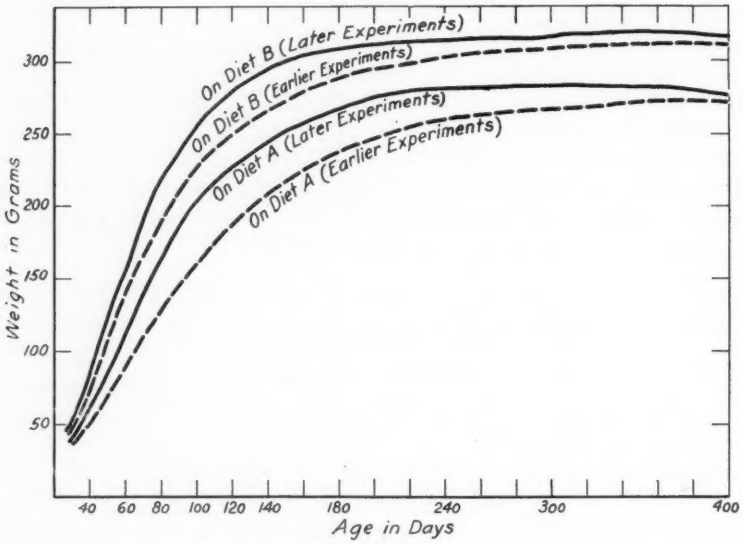

FIGURE 1

Weight curves of male rats.

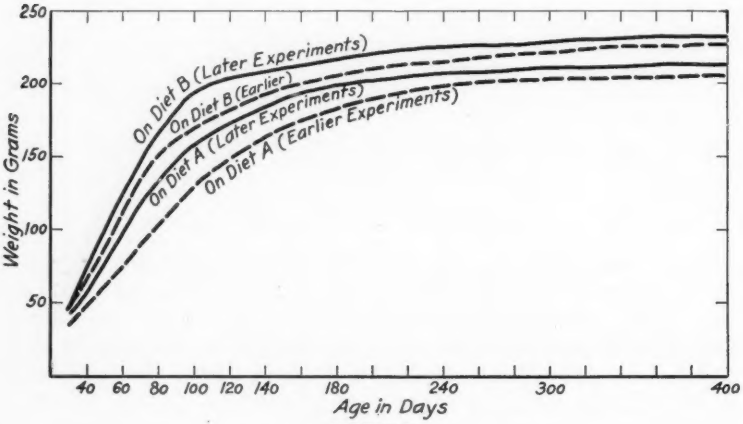

FIGURE 2

Weight curves of female rats.

ment, and slightly increased size, with evidences of greater vigor at all ages, and longer average life, when they received the dietary enriched as mentioned above. The average weight curves are shown in figures 1 and 2, and other averages are summarized in table 1. The food consumption in calories per day per unit of body weight was essentially the same for the two diets.

Summary.—Animals from families which were still thriving after eight generations on a dietary relatively poor in "protective" food were found capable of responding to enrichment of diet (in calcium, vitamins A and G, and to a less extent in protein) in the same manner previously found with animals taken at random from the same stock.

Among the evidences of increased nutritional well being following this enrichment of the food supply in some of its chemical factors were: (1) more rapid and efficient early growth; (2) somewhat larger average size at all ages, and this not attributable to higher food intake as expressed in calories per day per unit of body weight; (3) higher adult vitality as shown by earlier attainment and longer duration of reproductive capacity in the females, with rearing of both a larger number and a larger proportion of their young and to a larger average size during the suckling period; (4) increases of about ten per cent in the average lengths of life of adults of both sexes.

Thus the families which had become adapted to a relatively poor diet were, nevertheless, still capable of showing the same nutritional response to enrichment of chemical intake as previously described for test animals taken by random sampling with no such special background of dietary habituation.

The coöperation of the Carnegie Corporation of New York and the Carnegie Institution of Washington is gratefully acknowledged.

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THE ELASTIC PROPERTIES OF ROCKS: A CORRELATION OF THEORY AND EXPERIMENT*

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Introduction.—The theory of elastic-wave propagation in a homogeneous, isotropic, and infinitely extended medium provides the following well-known relations between the velocities of the waves and the four elastic constants of the medium:

$$V^2 = \frac{E}{\rho}\frac{1-\sigma}{(1+\sigma)(1-2\sigma)} = \frac{\mu}{\rho}\frac{4-E/\mu}{3-E/\mu} = \frac{3}{\rho\beta}\frac{3+E\beta}{9-E\beta} \quad (1)$$

$$v^2 = \frac{E}{\rho}\frac{1}{2(1+\sigma)} = \frac{\mu}{\rho} = \frac{E}{\rho}\frac{3}{9-E\beta} \quad (2)$$

There are also the connecting formulas relating the elastic constants to each other:

$$E\beta = 3(1-2\sigma) \quad (3)$$

$$E/\mu = 2(1+\sigma) \quad (4)$$

$$E\beta + 3E/\mu = 9 \quad (5)$$

$$\frac{\mu\beta}{3} = \frac{1-2\sigma}{2(1+\sigma)} \quad (6)$$

In these formulas

- E is Young's modulus of elasticity
- β is the cubic compressibility
- μ is the modulus of rigidity
- σ is Poisson's ratio of lateral contraction to longitudinal extension
- ρ is the density of the medium
- V is the velocity of a longitudinal wave in the medium
- v is the velocity of a transverse wave in the medium

Obviously formulas (1) and (2) can be extended to express V and v in terms of any two of the four constants, E, μ, β, σ.

The study of elastic waves in the earth's crust enables us to determine the longitudinal and transverse wave-velocities and the geometry of the wave path. If it be assumed that the formulas given above are valid for wave propagation in rocks, these data can be used to compute the elastic constants of the sub-surface layers. If these constants be compared with

values obtained by laboratory methods from samples of rocks subjected to the appropriate pressures and temperatures, it should be possible to identify the constituents of the crust at inaccessible depths.

This procedure meets with difficulty in practice. The assumption that the formulas of elastic theory can be applied to rocks requires experimental justification. It has never been demonstrated that rock masses occurring in Nature are sufficiently homogeneous and isotropic to provide the conditions which the theory requires. Secondly, the available laboratory data on the elastic properties of rocks as a function of hydrostatic pressure are inadequate to determine the effect of depth on the velocities of propagation. The compressibility[1] alone has been successfully measured in rock samples under pressure, while at least two of the elastic constants should be known in order to compute wave-velocities from the formulas without extrapolation.

The first of these difficulties, the question of the validity of the formulas when applied to rocks, has not received adequate discussion. Experimental measurements of the elastic constants of rocks have been few in number and sufficiently discordant to allow the assumption that discrepancies between theory and experiment could be charged entirely to limitations in the methods of measurement. The recent development of dynamic methods of measuring Young's modulus, Poisson's ratio and the modulus of rigidity in rock samples makes possible a more complete study of this question.

A thorough experimental test of the theoretical formulas requires laboratory measurements of the four elastic constants for a given set of rock samples, and seismic measurements of wave-velocities in the formations from which the samples are taken. A set of rock samples, prepared for the Committee on Geophysical Research at Harvard University, has been studied extensively with this object in view. W. A. Zisman[2] has published measurements of cubic compressibility, Young's modulus and Poisson's ratio, determined by static methods for these samples. The author[3] has published determinations of Young's modulus for the same and additional samples, using a dynamic method which simulates the conditions of wave propagation more adequately than the static method and hence gives more reliable values for the seismically effective modulus. The present paper describes measurements of the modulus of rigidity and Poisson's ratio for many of these samples, using dynamic methods. L. D. Leet[4] has published seismic determinations of longitudinal and transverse wave-velocities for three of the formations from which these samples were taken. When all of these data are correlated and compared with the theoretical formulas, it becomes possible to define the limits within which the theory for an ideal elastic medium applies to several important types of rock.

Petrographic descriptions of the rock samples may be found in the publications referred to above. E, μ and σ were measured on cylindrical samples, two inches in diameter and ten inches long. Two specimens, Sudbury norite No. 3 and No. 4, were $1^1/_8$ inches in diameter and eight inches long. Zisman's measurements of compressibility were made on smaller cores, $^5/_8$ inch in diameter and eight inches long. Wherever practicable a large and a small cylinder were cut from each block of rock along each of three mutually perpendicular axes, in order to study the variation of elastic properties with direction.

The methods of measurement of E, σ and β have been described in detail elsewhere. Zisman used a rocking-mirror piezometer to measure the yielding of the sample when its ends were subjected to compression in a testing machine. From the longitudinal yielding Young's modulus was determined, and from the lateral expansion Poisson's ratio was determined, for statically applied stresses up to 56 kg./sq. cm. He measured the compressibility by a modification of P. W. Bridgman's well-known lever piezometer, while the specimen was subjected to hydrostatic pressure transmitted through kerosene. The dynamic measurements of Young's modulus by the author were carried out by setting the specimen into vibration by electrostatic traction, and determining its natural frequency of longitudinal vibration, by observing the response of a piezoelectric crystal sound receiver, as the frequency of the driving voltage was varied through resonance. L. D. Leet's seismographic determinations of longitudinal and transverse wave-velocities were derived from experimental time-distance curves for explosion waves produced close to the surface in three formations: norite at Sudbury, Ontario; granite at Rockport, Massachusetts; and granite at Quincy, Massachusetts.

Dynamic Measurements of the Modulus of Rigidity in Rocks.—The new measurements presented in this paper consist of dynamically determined values of the modulus of rigidity and Poisson's ratio, for the set of rock samples described above. Values of the modulus of rigidity are computed from torsional resonance frequencies of the large-sized cores. Since this modulus is related to the natural frequency of torsional vibration, f_t, by the simple formula:

$$\mu = 4\rho l^2 f \int_t^2$$

it can be measured by any device for setting the rock cylinders into torsional vibration. The present method is to fasten a steel strip rigidly in a saw cut, at one end of the rock sample, and to produce torsion of the strip by two small electromagnets carrying alternating current from a variable-frequency oscillator. The electromagnets are set on opposite sides of the strip, and produce a torque, which is transmitted to the end

of the rock. A similar device at the opposite end of the sample, with the electromagnets connected to an amplifier, is used to detect the vibrations of the cylinder. The arrangement is a modification of one used by R. L. Wegel and H. Walther of the Bell Laboratories,[5] in a study of the vibrations of metal rods.

It is also possible to excite torsional vibrations piezo-electrically, by applying an alternating voltage across a small crystal of Rochelle salt. The crystal is cemented to a flange of sheet steel which projects eccentrically from one end face of the sample. A similarly placed crystal on the opposite end of the rock, connected to an amplifier, serves to detect the vibrations.

It is much more difficult to set up torsional oscillations than longitudinal oscillations, by either the magnetic or the piezo-electric method. The accuracy of these methods is limited by the loading of the steel pieces cemented to the specimen ends. This effect introduces uncertainties of the order of $^1/_2$ per cent.

The modulus of rigidity, determined from these measurements of torsional frequencies, is listed for thirty rock samples in the second column of table 1. So far as the author is aware, these are the first published measurements of the rigidity of rocks under extremely minute stress. A discussion of these data will be deferred until we have described the method of measuring Poisson's ratio.

Dynamic Measurements of Poisson's Ratio in Rocks.—Poisson's ratio is generally conceded to be the most difficult of the elastic constants to measure, yet it seems worth while to supplement Zisman's careful static determinations of this ratio with some dynamic measurements on the same samples. The method used is one suggested by E. Giebe and E. Blechschmidt,[6] and tested by them on magnetostrictive rods of nickel and invar. This consists of finding the spectrum of the harmonic longitudinal resonance frequencies of the cylinder, to as high an order as the sensitivity of the vibration detector permits. As is well known, the frequency intervals between successive harmonics become smaller as the order of the harmonic increases. This is caused by the absorption of energy from the longitudinal vibrations by radial motion. The radial resonance frequency, determined by the diameter of the cylinder, is coupled to the longitudinal wave system and produces the observed distortion of the latter. The coefficient of coupling between the radial and longitudinal systems of vibration is a function of Poisson's ratio, which can be obtained graphically by appropriate plotting of the data.

In practice, this method of measuring Poisson's ratio is incapable of more than about ten per cent accuracy, on account of the irregularity of the harmonic frequencies obtainable from even the best of the rock specimens. Since there are experimental limitations of this magnitude, it

seems inadvisable to devote much space to the mathematical theory of the method. The reader is referred to the papers of Giebe and Blechschmidt,[6] L. Posener[7] and R. Ruedy[8] for a complete treatment of the propagation of longitudinal waves in cylindrical rods.

A convenient means of deriving Poisson's ratio from the experimental data is to plot F^2 against $F^4/(K^2f_0^2 - F^2)$ for each sample. Let F represent the observed frequency sequence, K the order of the harmonic, f_0 the fundamental longitudinal frequency and f_r the radial resonance frequency. If Kf_0 and f_r are the two undistorted frequencies which are coupled together with the coefficient of coupling, q, it can be shown from the theory of coupled systems that:

$$(K^2f_0^2 - F^2)(f^2 - F^2) = q^2F^4.$$

This can be written:

$$F^2 = -q^2F^4/(K^2f_0^2 - F^2) + f_r^2.$$

Now if F^2 be plotted against $F^4/(K^2f_0^2 - F^2)$ the result is a straight line with slope q^2 and intercept f_r^2. It can be shown that for cylindrical tubes, $q = \sigma$, so that Poisson's ratio is the square root of the slope of the plotted line. For cylindrical rods the theory is more complicated, but the ratio can be evaluated from q by means of Bessel's functions. The relation between q and σ is given numerically in the following table, reproduced from the article of Giebe and Blechschmidt:

q	0.556	0.493	0.396	0.359	0.304	0.190
σ	0.33	0.30	0.25	0.23	0.20	0.13

The mathematical analysis of longitudinal frequencies in terms of coupled systems is rigorous for tubes, but must be regarded as an approximation for cylinders. The results of Giebe and Blechschmidt for metal rods, however, indicate that the approximate theory represents the observed effects within experimental error, in the range of frequencies below f_r.

Figure 1 shows F^2 plotted against $F^4/(K^2f_0^2 - F^2)$ for steel, copper, diabase, slate, quartzitic sandstone, granite and marble. The order of the harmonic, K, is given by the number opposite each plotted point. In each case the slope of the line joining the points is q^2, from which Poisson's ratio may be determined by interpolation in the table given above.

It is found that the values of Poisson's ratio obtained by this method agree within five to ten per cent with those computed by means of formula (4) from Young's modulus and the rigidity. Since the harmonic series method is only accurate to approximately ten per cent, we conclude that within the limits of experimental error this method gives the same result

TABLE 1

MODULUS OF RIGIDITY, COMPRESSIBILITY AND YOUNG'S MODULUS IN REPRESENTATIVE AMERICAN ROCKS

MATERIAL	NO.	MODULUS OF RIGIDITY $\mu \times 10^{-11}$ DYNES/CM.^{2}	COMPRESSIBILITY $\beta \times 10^{12}$ CM.^{2}/DYNE	YOUNG'S MODULUS $E \times 10^{-11}$ DYNES/CM.^{2}	$E\beta + \frac{3E}{\mu}$ NUMERIC	DEVIATION FROM 9 PER CENT
Granite						
Quincy Surface	1	1.97				
Quincy Surface	2	1.80	24.7	3.95	7.28	−19.1
Quincy Surface	3	2.27				
Quincy Surface	7	1.55				
Quincy 100′ deep	1	2.78				
Quincy 100′ deep	2	2.77	19.2	5.99	7.69	−14.6
Quincy 100′ deep	3	2.70				
Quincy 235′ deep	4	2.07				
Quincy 235′ deep	5	1.87	21.1	4.72	7.87	−12.6
Rockport 100′ deep "hardest way"		1.71				
Rockport 100′ deep "easiest way"		2.00	19.5	4.51	7.87	−12.6
Westerly		1.89	..	3.99	..	....
Olivine Diabase						
Vinal Haven	1	4.21				
Vinal Haven	2	4.19	14.6	10.58	9.12	+ 1.3
Vinal Haven	3	4.17				
Norite						
Sudbury	3	3.60				
Sudbury	4	3.89	16.5	8.90	8.79	− 2.3
French Creek	2	3.07				
French Creek	3	3.54	14.0	7.88	8.27	− 8.1
Slate						
Pennsylvania		4.65	..	11.29	..	....
Quartzitic Sandstone						
Pennsylvania	1	3.24				
Pennsylvania	2	3.10	26.7	6.40	8.15	− 9.4
Pennsylvania	3	2.62				
Gneiss						
Pelham (perpendicular to foliation)	1	1.45	20.7	2.70	6.44	−28.4
Dolomite						
Pennsylvania	1	3.23				
Pennsylvania	2	3.62	11.9	8.52	8.09	−10.1
Pennsylvania	3	3.98				
Marble						
Vermont ($\parallel$ to bedding)	1	2.17				
Vermont ($\perp$ to bedding)	2	1.14	13.9	4.28	7.61	−15.5
Vermont ($\parallel$ to bedding)	3	2.23				
Copper		4.59	7.6	12.5	9.11	+ 1.2
Steel		8.38	6.0	21.3	8.90	− 1.1

for Poisson's ratio as is obtained by combining the dynamic measurements of E and μ. Consequently the data from the harmonic series method are not separately listed in tables 1 and 2, but are used to supplement the more accurate data from torsional frequencies in cases where the latter

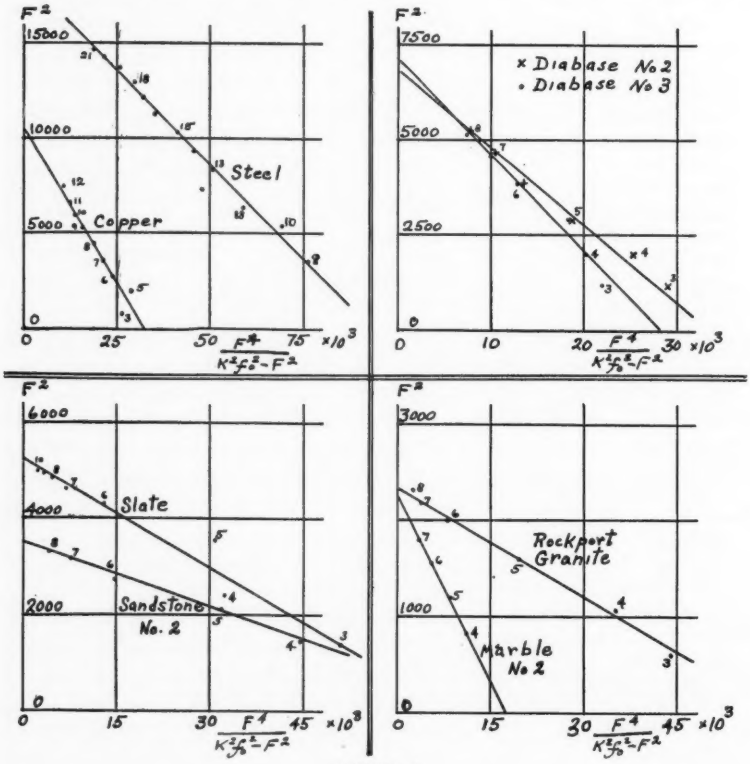

FIGURE 1

The series of longitudinal harmonic frequencies of a rock or metal cylinder, yields a straight line when plotted in the above form. From the slope of this line Poisson's ratio may be determined.

are incomplete. For example, the marble samples give longitudinal harmonics as far as the seventh, while the torsional frequencies cannot be detected, because, perhaps, of the high damping in this material. The principal value, however, of the harmonic data lies in the fact that they agree with the results of the other dynamic methods. By formula (4) from elastic theory, E/μ can be computed to two per cent, if σ is known to ten per cent. Within these limits the formula is thus experimentally

verified by separate dynamic determinations of E, μ and σ. This verification, although limited in precision, is of value in view of the complicated theory underlying the harmonic method, and it increases confidence in the relatively simple theory at the basis of our measurement of Young's modulus and the rigidity.

This completes the presentation of the new measurements of the rigidity and Poisson's ratio obtained for the rock samples from torsional resonance frequencies, and from longitudinal harmonic frequencies, respectively. We are now in a position to correlate these measurements with those described in previous publications, for Young's modulus, cubic compressibility and transverse and longitudinal wave-velocities. The object of the correlation is to establish the experimental limits within which the formulas from elastic theory may be expected to be valid.

Correlation of Young's Modulus, Rigidity and Compressibility of Rocks.— In order to compare the experimental data with formulas (1) to (6), we have grouped them in several different ways. First, formula (5) is selected for detailed discussion, since it expresses the relationship between E, β and μ, the three elastic constants which are most reliably determined by experiment. The values of these constants and the comparison with formula (5) appear in table 1. Next, we compare five different methods of determining Poisson's ratio. The material in table 2 and formulas (1), (2), (3) and (4) are used for this discussion. Finally, we compare formulas (1) and (2) with experiment, by computing longitudinal and transverse wave-velocities from measured values of E, μ and β. The computed and observed wave-velocities are compared in table 3.

Table 1 summarizes the experimental determinations of μ, β and E, for thirty samples of rock and for copper and steel (atmospheric pressure). The rigidity measurements are described above, the values of compressibility are quoted from W. A. Zisman, and the dynamically determined values of Young's modulus are quoted from a previous publication by the author.

In the last two columns of table 1 the values of E, β and μ are combined in accordance with formula (5) from elastic theory, which states that $E\beta + 3E/\mu = 9$. Column five gives the sum of the two left-hand terms, and column six gives the percentage deviation of this sum from nine. It is evident from the table that the experimental data fit the formula with varying degrees of accuracy. For copper and steel, and for the diabase rock, there is agreement between theory and experiment to one per cent. Elastic properties can seldom be measured more accurately than this. For rocks which are less compact than the diabase, more porous or composed of larger crystals, the formulas do not describe the observed effects as well. The norite, sandstone, dolomite, marble and granite show disagreement increasing from two per cent to nineteen per cent. The sample

TABLE 2

POISSON'S RATIO FOR ROCKS, DETERMINED BY THREE METHODS

MATERIAL	NO.	BY STATIC MEASUREMENT	BY FORMULA: $\sigma = \frac{1}{2}\left(\frac{E}{\mu} - 2\right)$	FORMULA: $\sigma = \frac{1}{2}\left(1 - \frac{E}{3\beta}\right)$
Granite				
Quincy Surface	1	...	0.055	
Quincy Surface	2	...	0.045	
Quincy Surface	3	...	0.040	0.338
Quincy Surface	7	...	0.048	
Quincy 100′ deep	1	...	0.073	
Quincy 100′ deep	2	...	0.069	0.309
Quincy 100′ deep	3	...	0.118	
Quincy 235′ deep	4	...	0.130	0.333
Quincy 235′ deep	5	0.117	0.160	
Rockport 100′ deep "hardest way"		0.106	0.180	0.354
Rockport 100′ deep "easiest way"		0.097	0.144	
Westerly		...	0.065	
Olivine Diabase				
Vinal Haven	1	0.271	0.270	
Vinal Haven	2	0.258	0.260	0.244
Vinal Haven	3	0.275	0.259	
Norite				
Sudbury	3	0.236	0.230	
Sudbury	4	0.224	0.210	0.205
French Creek	2	0.125	0.160	
French Creek	3	0.152	0.230	0.317
Slate				
Pennsylvania		...	0.210	
Quartzitic Sandstone				
Pennsylvania	1	0.112	0.105	
Pennsylvania	2	...	0.025	0.215
Pennsylvania	3	...	0.088	
Gneiss				
Pelham (perpendicular to foliation)	1	0.030	−0.020	0.407
Dolomite				
Pennsylvania	1	...	0.100	
Pennsylvania	2	...	0.285	0.330
Pennsylvania	3	...	0.150	
Marble				
Vermont ($\parallel$ to bedding)	1	0.166	0.138	
Vermont ($\perp$ to bedding)	2	0.146	0.226	0.400
Vermont ($\parallel$ to bedding)	3	0.185	0.144	
Copper		...	0.370	0.342
Steel		...	0.270	0.287

of gneiss, cut perpendicular to the foliation, shows almost thirty per cent deviation from the formula.

Another conclusion we may draw from the tabulated comparison is that the disagreement between theory and experiment is systematic in direction, that is, the experimental value of $E\beta + 3E/\mu$ is uniformly smaller than we should expect. The quantity $E\beta$ is in general less than twenty per cent of the sum of the two terms of the formula, so that an experimental error of twelve per cent in the determination of the compressibility results in only two per cent change in the left side of the equation. The bulk of the observed discrepancy must, therefore, be charged to the second term, $3E/\mu$. This term is proportional to the ratio of longitudinal to torsional resonance frequencies, squared. It is thus obtained directly from experiment to a precision of about $^1/_2$ per cent. Alternatively E/μ can be computed by means of formula (4), using the data for Poisson's ratio from harmonic series, or the statically determined values

TABLE 3

COMPARISON OF FIELD AND LABORATORY DETERMINATIONS OF ELASTIC-WAVE VELOCITIES IN ROCKS

A and A' Computed from Young's Modulus and Rigidity.
B and B' Computed from seismic measurements.
C and C' Computed from Young's Modulus and Compressibility.

ROCK	TRANSVERSE WAVE VELOCITY v IN KM./SEC.		DIFFERENCES $\frac{A-B}{B}$ AND $\frac{C-B}{B}$	LONGITUDINAL WAVE VELOCITY V IN KM./SEC.		DIFFERENCES $\frac{A'-B'}{B'}$ AND $\frac{C'-B'}{B'}$
Sudbury Norite	A	3.57		A'	5.93	
			+ 2.3%			− 4.7%
	B	3.49		B'	6.22	
			+ 1.2			− 1.0
	C	3.53		C'	6.16	
Rockport Granite	A	2.66		A'	4.18	
			− 1.5			−18.7
	B	2.70		B'	5.14	
			− 6.7			+ 2.9
	C	2.52		C'	5.29	
Quincy Granite	A	2.93		A'	4.28	
			+18.1			−13.7
	B	2.48		B'	4.96	
			+ 5.6			+ 3.6
	C	2.62		C'	5.14	

given by Zisman. All three of these groupings of experimental data agree in giving smaller values of E/μ than we should expect from formula (5). This indicates that the discrepancies listed in column seven of table 1 are not chargeable to limitations in the methods, but are characteristic of the materials when measured at atmospheric pressure.

We conclude from the data of table 1 that formula (5) agrees with

experiment for metals and for diabase, but that experimental values of $E\beta + 3E/\mu$ may be from ten to thirty per cent lower than the formula predicts for such rocks as marble, quartzitic sandstone, dolomite, granite and gneiss. We can also make a reasonable guess that disagreement between theory and experiment will be still larger for the less compact sedimentary rocks such as shales, conglomerates and soft sandstones.

Comparison of Values of Poisson's Ratio by Five Methods.—Another comparison between theory and experiment is made in table 2. Here are listed values of Poisson's ratio obtained by three methods: (*A*) static measurement (quoted from data of W. A. Zisman), (*B*) computation by means of formula (4) from dynamic measurement of E/μ, and (*C*) computation by means of formula (3) from E and β. The table gives average results for several samples of each type of rock, at atmospheric pressure.

The second and third columns of table 2 enable us to compare static and dynamic methods of measuring Poisson's ratio. It is evident that agreement is reasonably good only for diabase and Sudbury norite. For the other rocks there is a tendency for the statically determined value to be smaller than the dynamically determined value, and the difference between them is as large as thirty per cent in some cases.

When Poisson's ratio is computed by means of formula (3) from the measured values of Young's modulus and compressibility, we find still larger discrepancies. Again there is substantial agreement for diabase and Sudbury norite, but for the other rocks the formula gives values higher by a factor of two or three than are obtained by either of the other methods. It is also apparent from table 2 that the larger the value of Poisson's ratio from formula (3), the smaller will be the value computed from formula (4). For example, for gneiss, formula (3) gives $\sigma = 0.407$, while formula (4) gives $\sigma = -0.02$. These are respectively the largest and the smallest values obtained.

A fourth method of determining Poisson's ratio is from formulas (1) and (2), using Leet's values of longitudinal and transverse wave-velocity. Data are available for three formations: (1) Sudbury norite, for which $\sigma = 0.27$, (2) Rockport granite, for which $\sigma = 0.31$ and (3) Quincy granite, for which $\sigma = 0.33$. Comparison with the values in table 2 shows that in each case agreement with the computation from formula (3) is surprisingly good.

A fifth method of measuring Poisson's ratio, employing the departure of the longitudinal harmonic frequencies from integral multiples of the fundamental, is discussed above and its results found to agree within experimental error with those computed from E/μ.

We have thus determined Poisson's ratio by five methods, involving widely different principles. Of these, the two laboratory dynamic methods agree in giving relatively low values, e.g., $\sigma = 0.09$ for Quincy

granite. The seismic method agrees with formula (3) in giving relatively high values, e.g., $\sigma = 0.33$ for Quincy granite. The static method gives values which are discordant, but in general even lower than those derived from dynamic measurements.

There seems to be no possibility of experimental errors large enough to explain these general results, although minor discrepancies may be accounted for in this way. We must, therefore, conclude that the large discrepancies arise from the use of formulas which are inadequate to describe the actual elastic behavior of these rocks. A reasonable explanation is that most rocks are not sufficiently homogeneous and isotropic to meet exactly the conditions under which the formulas are derived.

The discrepancies between the values of Poisson's ratio from these various formulas are quantitatively much larger than the divergences between theory and experiment listed in table 1 for formula (5). This is true because of the manner in which σ enters into formulas (1), (2) and (4). For example, a ten per cent variation in σ produces only two per cent variation in E/μ computed from formula (4). Similarly a ten per cent variation in σ produces only 3.7 per cent variation in V/v from formulas (1) and (2). But aside from the quantitative difference between the discrepancies of table 1 and of table 2, the general results are very similar. If the various types of rock are arranged in order of increasing deviation between theory and experiment, this order is the same for each of the various groupings of computed and observed data. With some overlapping the sequence is: diabase, norite, quartzitic sandstone, dolomite, marble, granite and gneiss. Two types of rock which figured in earlier papers on this set of samples are not discussed here. These are limestone from Pennsylvania and periodotite from North Carolina. The elastic frequency spectra of these rocks are so irregular that torsional frequencies cannot be identified, nor can the harmonic series be used to determine Poisson's ratio. The irregularities are probably due to internal cracks of sufficient size to prevent the formation of regular standing-wave systems when the cylinders are dynamically stressed.

Comparison of Observed and Computed Wave-Velocities in Rocks.—For the Sudbury norite, Quincy granite and Rockport granite, we can compare computed wave-velocities with Leet's direct field measurements of these constants (maximum pressure a few atmospheres). This comparison is of particular interest since wave-velocities are the data of primary concern to seismologists.

Table 3 contains values obtained in three ways: (*A*) computed by formulas (1) and (2) from laboratory determinations of Young's modulus and the modulus of rigidity; (*B*) obtained by Leet from time-distance curves for explosion waves; and (*C*) computed by formulas (1) and (2) from laboratory measurements of Young's modulus and compressibility.

The primed letters in the table indicate longitudinal wave-velocities, and the unprimed letters indicate transverse wave-velocities. The percentage differences between computed and observed wave-velocities are also tabulated. Each laboratory determination is an average for several rock cores, while each field measurement represents as average of seismograph records taken at intervals along a contour several thousand feet long.

It is evident from the table that the computations in terms of $E\beta$ check with experiment much more closely than those in terms of E/μ. It is also evident that the agreement for Sudbury norite is much better than for either of the granites. Both conclusions are entirely consistent with the comparisons from table 1 and table 2.

Summary.—Dynamic measurements of the modulus of rigidity of rocks are described. These are computed from the torsional resonance frequencies of cylindrical samples.

Dynamic measurements of Poisson's ratio are discussed. These are obtained from the deviation of the longitudinal harmonic frequencies from integral multiples of the fundamental, and are found to agree with values computed from Young's modulus and the modulus of rigidity.

The relations between the elastic constants determined in the laboratory and the theoretical formulas, which express the connections between the constants of a perfect elastic medium, are discussed in detail. It is found that $E\beta + 3E/\mu$, computed from experimental values, is smaller than the theoretical formula predicts, for all the rocks investigated except diabase. There is agreement between theory and experiment to one per cent for diabase, copper and steel. It appears that the values of Poisson's ratio determined from E/μ, static measurements and harmonic series, are relatively much lower than those calculated by means of the formulas involving $E\beta$ or V/v. It is found, finally, that field measurements of elastic-wave velocities in the Sudbury norite agree to approximately one per cent with velocities computed from $E\beta$, and to approximately five per cent with velocities computed from E/μ. The check with field determinations for granite is within seven per cent for velocities computed from $E\beta$, and within nineteen per cent for velocities computed from E/μ.

Conclusion.—The reliability of the laboratory methods employed in this research is established by the excellent agreement with theory obtained for steel, copper and diabase. Thus we must conclude that the discrepancies found are real, and that most of the rocks studied are, therefore, imperfect elastic media. The theoretical formulas are derived subject to the condition that the medium is homogeneous, isotropic and perfectly elastic. It is certainly reasonable that some types of rock are more adequately described than others in these terms. Experiment shows this to be true, and gives the sign and order of magnitude of the discrepancies to be expected.

All of the comparisons between theory and experiment agree that experimental values for the modulus of rigidity are too large, and experimental values of Poisson's ratio are too small, to give good agreement when substituted in the various theoretical formulas. Better agreement is obtained when neither of these constants is involved, as when wave-velocities are computed from experimental values of Young's modulus and compressibility.

We have shown from measurements on rock samples at atmospheric pressure, that the theoretical formulas cannot be used to compute one elastic constant from the experimental determination of another, without running the risk of making large errors. The risk varies with the formulas compared and with the type of rock under discussion. But as long as this uncertainty exists, the comparison of laboratory and field measurements of elastic constants cannot be regarded as a reliable means of identifying the constituents of the earth's crust. It is possible that if the elastic constants of rock samples could be measured at the pressure which exists at even a few hundred feet depth, theory and experiment might agree more closely. This seems reasonable, but until such agreement is found by experiment, we can have little confidence in wave-velocities computed from measurements on compressibility under hydrostatic pressure. It is obvious that determinations of compressibility must be supplemented by experiments on the other elastic constants under pressure, if their geophysical significance is to be established.

It is possible that dispersion, or a variation of velocity with frequency, exists in rocks to a sufficient degree to explain part of the discrepancy between laboratory and field measurements. This seems doubtful in view of the fact that static and dynamic measurements, at zero frequency and at 10,000 cycles per second, respectively, agree in giving values of E/μ and of Poisson's ratio much lower than are computed from Leet's determinations of transient group-velocities.

Another possibility is that viscosity effects, neglected in the derivation of the formulas, may be of sufficient magnitude in rocks like granite to account for part of the divergence between theory and observation.

These questions are vital to the solution of important problems in geophysics, but they can be answered only by further experimental work on the elastic properites of rocks under conditions comparable to those which exist below the surface of the earth.

In conclusion, the experiments described and correlated in this report suggest caution in applying the theory of elasticity to the surface rocks of the earth's crust, whether they be sedimentary or granitic in character. On the other hand, the results for diabase indicate that the theory can be applied with confidence to the fine-grained, igneous rocks which are presumed to lie beneath the oceans and the continental granites.

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THE UNDERLYING CAUSES OF SUBMARINE CANYONS

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Read before the Academy, April 27, 1936

As more and more deep submarine canyons are discovered off the various coasts of the world it is quite natural that much skepticism should be aroused relative to their origin as stream-cut valleys. Alternative modes of origin not involving large scale movements of the continental borders have been suggested recently by two of the foremost American geologists. The late Professor W. M. Davis[1] called attention to the possibility that winds sweeping water into a bay might set up a return current underneath which would keep open a valley-like depression while sediments were accumulating on either side. Professor Daly[2] on the other hand suggested that during the low sea level stages of the glacial period the waves might have attacked the mud banks of the outer continental shelves and that the resulting heavy muddy water would have moved down the continental slopes excavating submarine canyons where the flow became concentrated.

During recent years the writer has been given splendid coöperation from many organizations[3] in the exploration of these marine canyons and during this work has made every attempt to check the possibility of origins other than fluviatile. The large accumulation of data which has been acquired appears to have much bearing on the subject. To summarize this information: In the first place the canyons which have been surveyed in great detail are proving to have a close resemblance to river canyons on land. For example, it is perfectly obvious that a canyon with tributaries like the one shown in figure 1, which is from a model of the Monterey Bay submarine canyon, is the type of feature which rivers cut on land,

but quite different from the small channels and depressions which are definitely known to be due to submarine current scour. Also, the huge branching canyons with their axes shown in figure 2 are certainly of a fluviatile pattern.

In the second place the suggestions of possible current origin seem to have been made with the idea that the canyons penetrated soft sediments, but the exploration by the writer of 14 canyons on the west coast has shown that 11 have rocky walls, some with hard rock, even solid granite. In the investigations of H. C. Stetson off the east coast rock was also found in two canyons. It seems impossible that the narrow canyons with rocky

FIGURE 1
Model of Monterey Submarine Canyon constructed by Blackstone Model Company.

walls found off California could have been formed by ocean currents. Also, it is beyond all reason to suggest that a canyon like the one in figure 3 which compares so well with the Grand Canyon could be cut by the feeble submarine currents which are all that the dynamic oceanographers consider possible out in the open ocean. Furthermore, both direct measurement of currents and study of water samples from the canyons give a decided negative to the current hypothesis so far as present information goes. Finally, the penetration of numerous canyons right up to the coast does not allow the application of Daly's hypothesis in many cases, nor does the coastal configuration permit the use of Davis' idea in most cases. Despite these objections we should make further investigations of currents, particularly to see if they have not been a factor in keeping open canyons which were originally cut by rivers.

Since it seems evident that the canyons are not the product of current action and since it is obvious that they have shapes quite unlike those of diastrophic depressions, there appears to be no alternative but to believe that at least the American canyons were cut by rivers. To what extent

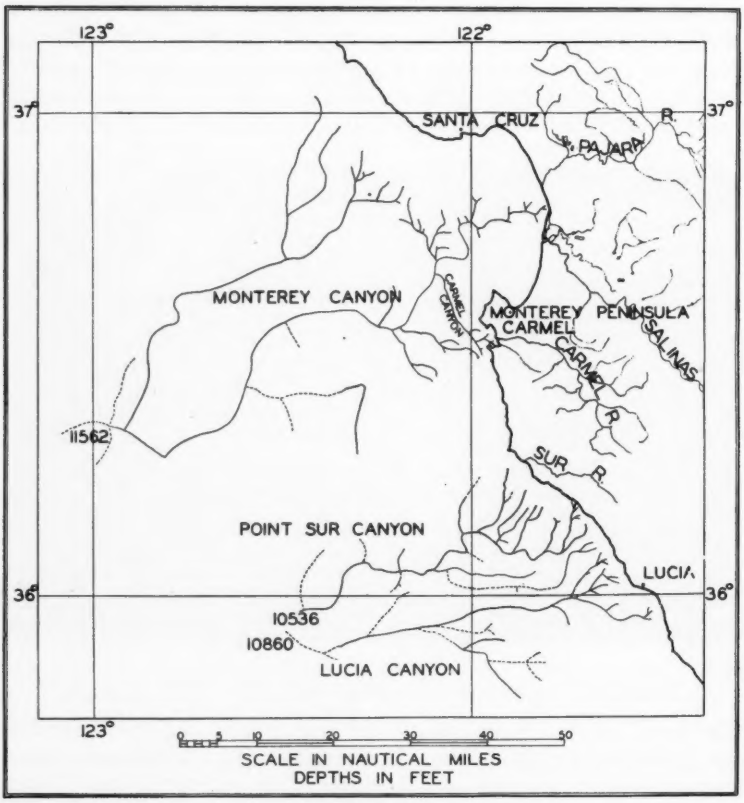

FIGURE 2

The trends of the canyons along the California coast from Lucia to Santa Cruz. Depths given in feet.

the same origin can be applied to the hundreds of canyons found around the various other coasts of the world can be decided only after more soundings have been made, but in the mean time the available evidence favors subaerial erosion for the marine canyons of the world.

From this conclusion we must infer that all or practically all of the continental borders stood higher in relation to the sea during some part of the

geological past in order to allow rivers to entrench themselves into these margins. This might mean that the margins were uplifted some time during the past, the canyons cut, and then the margins depressed. However, this sequence is hard to defend for the following reasons: First, such uplifts and subsidences could scarcely be expected to be as universal as would have been necessary. Second, the canyons are as well developed off what appear to be stable coasts as off the mobile coasts such as California. Third, the canyons appear both off coasts which have been sub-

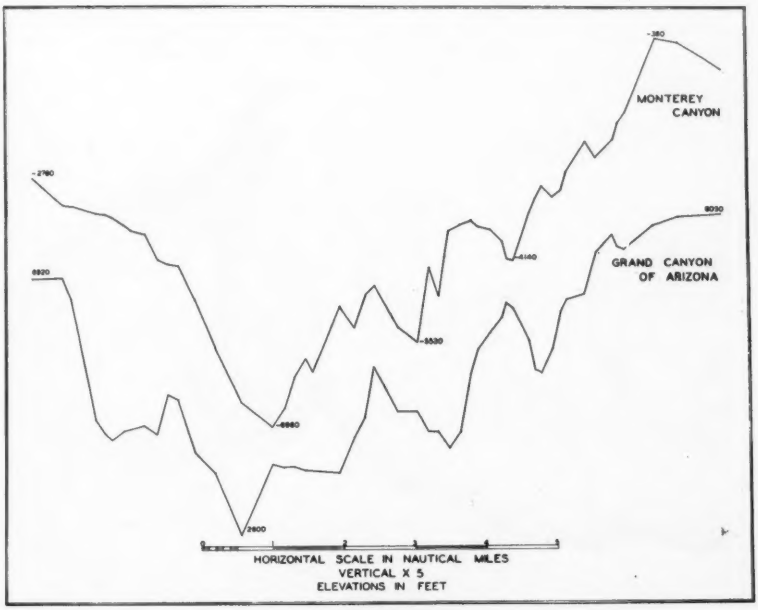

FIGURE 3

Section of Monterey Submarine Canyon compared to the Grand Canyon. Note that the same scale and the same spacing of observation points is used.

siding for long periods receiving large thicknesses of sediment and off coasts where uplift is indicated by wave-cut terraces. Fourth, the rocks dredged from the walls of some of the canyons contain fossils or stratigraphic indications of being late Tertiary or Pleistocene in age, but there is no evidence of sediments carried inland from any recently elevated margins.

Another hypothesis which would at least account for the universality of the submarine canyons is that the ocean basins sank drawing the water off the lands and then presently returned to their former position drowning

the canyons. This idea also has serious drawbacks. In the first place, the oceanic islands, particularly the coral islands, have abundant evidence of having been involved in the general withdrawal of the ocean waters. Secondly, it would seem to be mechanically impossible for the ocean basins to undergo such sinking. There is no place for the rock beneath the basins to go, and shrinkage of volume enough to account for thousands of feet of sinking is inconceivable. Thirdly, the reversal of the movement offers still more formidable difficulties.

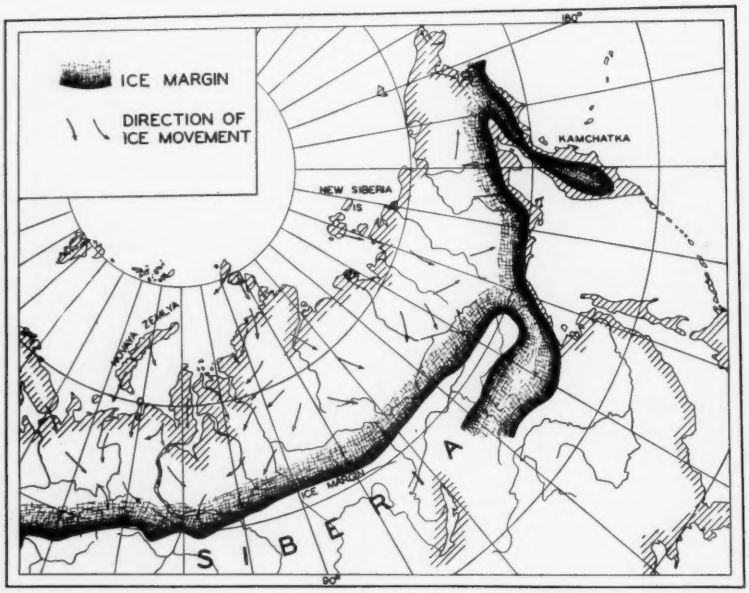

FIGURE 4

A map showing the direction of the ice movement in Northern Russia and Siberia based on a recent Soviet publication.

Another hypothesis which at least is not open to the various objections of the previous suggestions is that the level was changed due to formation of enormous ice caps on the land which would allow the cutting of valleys on the sea floor to the extent that the sea level had been lowered. The effects of such a change would be as great on the islands of the ocean basins as on the continental borders. Also, there is a mechanism here to account for the lowering of sea level to produce the canyon cutting, and for the canyon submergence. Furthermore, the time of lowering fits in with the

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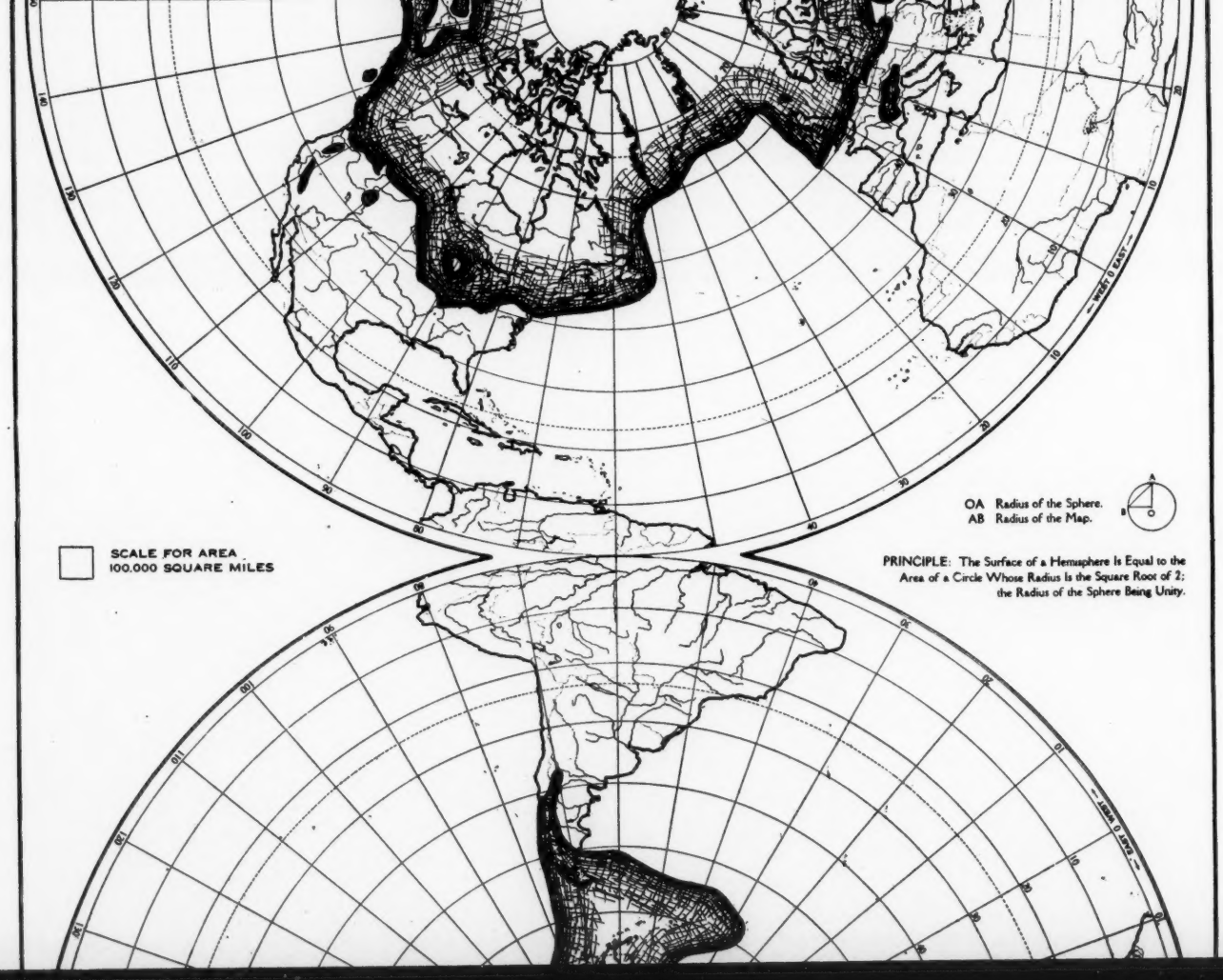
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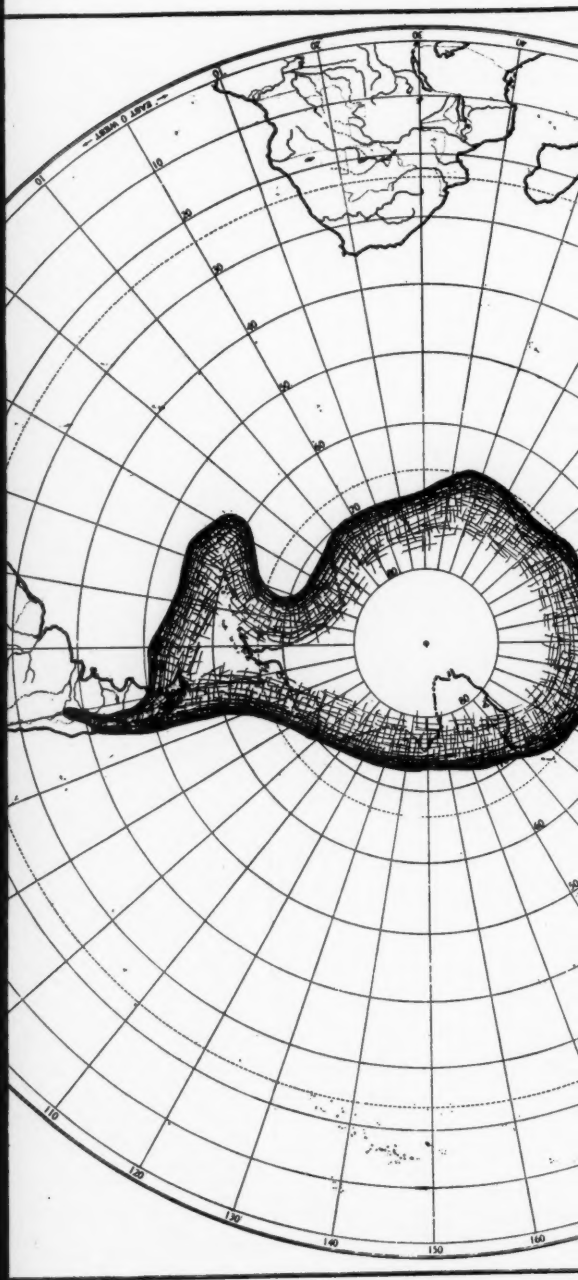
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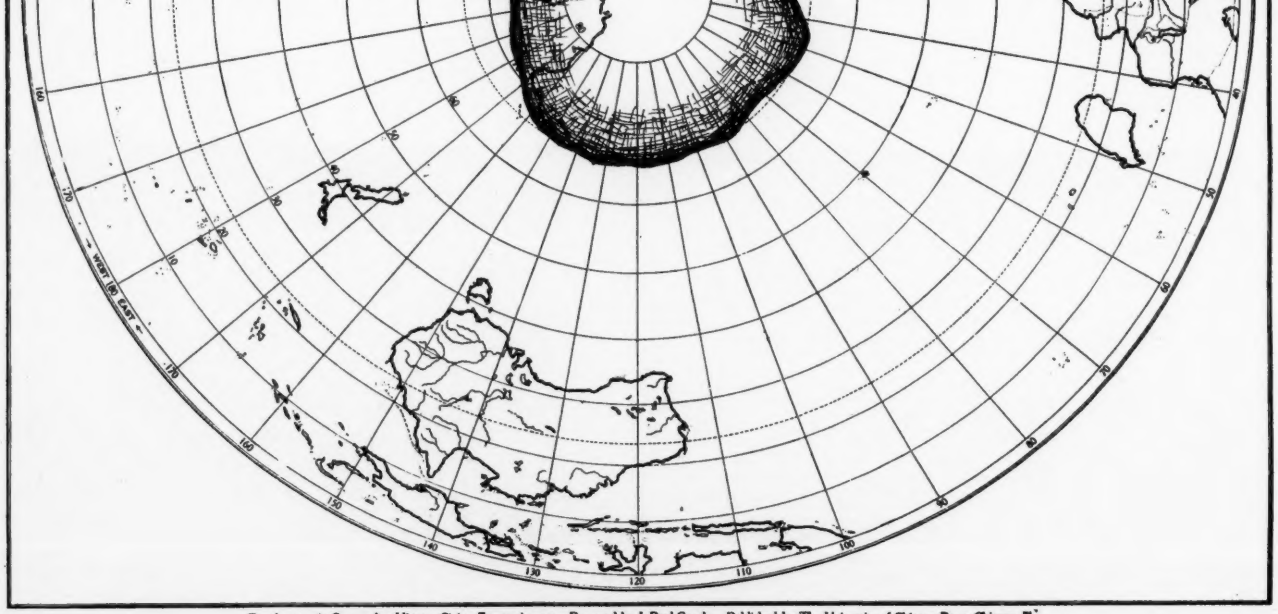

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FIGURE 5

Map showing the possible distribution of ice caps during the maximum glaciation of some early epoch.

available evidence as to the time of excavation at least of the inner canyons, namely, during the middle or early part of the glacial period.

The first problem raised by the glacial lowering of sea level is whether the ice could have been large enough in volume to account for it. There is no use in trying to claim that most of the ocean was piled on the lands lowering the sea level over ten thousand feet and leaving only a greatly shrunken and very salty ocean. On the other hand let us see if we cannot obtain some help from a less radical lowering. Common estimates of the reduction of sea level during the glacial period are of the order of 300 feet. These estimates, however, have been made with extreme conservatism. An average thickness of all the ice caps of only 3500 feet has been allowed,[4] but we know that near the margin of the American ice cap there was over 6000 feet of ice to cover Mt. Washington, and also that the ice in central Greenland is even now about 7000 feet thick. The estimates of the area covered by the ice caps may also be much too small, particularly in regard to the earlier glacial epochs. New evidence suggests that the Arctic ocean may have supported an ice cap starting as shelf ice and gradually sealing in the Arctic basin and building a dome on top of it. The thickening of the shelf ice would have caused it to ground on the relatively shallow margins of the Arctic basin, particularly as the seal level became lowered by growth of ice elsewhere. Figure 4 is based on a recent Soviet publication[5] and is one of several lines of evidence favoring the Arctic ice cap. At the same time the Antarctic ice cap may have grown and extending seaward as shelf ice it could have spread across Drake Strait forming a continuous cap as far north as South America. Possibly it may have extended to other continents although the deep intervening oceans would have greatly interfered. If the ice covered the area shown in figure 5 and had a thickness of 4 miles which is physically and meteorologically reasonable the sea level would have been lowered some 3000 feet. A still greater area and thickness of ice is possible, but even the 3000 foot estimate would account for the universality of canyons and for the indications of submergence of the oceanic islands. It does not, however, account for the deeper parts of the canyons.

The following suggestions are the best that the writer can offer at present as a general explanation: First, that prior to the glacial period there were various depressions on the continental slopes which were in part the result of landslides or mudflows, in part diastrophic, and in part true river canyons submerged by diastrophism. Second, that the sea level was lowered 3000 feet or more during the maximum glaciation of some early epoch and that the rivers from the land flowed into the various preëxisting depressions and cut true river canyons through relatively recent sediments connecting these canyons with the deep outer depressions. Third, that the sea level rose with the melting of the ice and that the canyons have been

maintained ever since on the steep slopes due to the mud flows which are still occurring from time to time and that currents may have played some part in keeping the canyon heads clear of sediment.

[1] W. M. Davis, "Submarine Mock Valleys," *Geog. Review*, **24**, 297–308 (1934)

[2] R. A. Daly, "Origin of Submarine 'Canyons,'" presented before Geological Society of America, December 26 (1935).

[3] Grants for this work have been made by The Geological Society of America and the NATIONAL RESEARCH COUNCIL. Also, much assistance has been given by the Coast and Geodetic Survey, The Scripps Institution, The Hopkins Marine Station and many other organizations and individuals.

[4] R. A. Daly, *Changing World of the Ice Age*, p. 46 (1934).

[5] P. T. Stepanov, "Useful Minerals of the North U. S. S. R.," **1**, *Geology*, Moscow (1935) (in Russian).

ON THE MECHANISM OF INHIBITION OF CRAYFISH MUSCLE

BY C. A. G. WIERSMA AND GEORGE MARMONT

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Communicated July 2, 1936

Recent work in this laboratory has demonstrated the distribution and the function of the various motor and inhibitory axons innervating the muscles of the cheliped of Cambarus clarkii. For instance, it was found that the adductor of the dactylopodite is innervated by two motor fibres, giving rise respectively to a twitch and to a slow contraction[1]; that one motor fibre innervates both the abductor of the dactylopodite and the extensor of the propodite, but that each of these muscles has its own inhibitory fibre[2]; and that the flexor of the propodite is triply innervated, having a fibre giving rise to a "fast" and one to a "slow" contraction and a third one stimulation of which can inhibit both these contractions.[2] These facts have been used to carry out experiments on the mechanism of inhibition. This paper is a preliminary note on this work.

Method.—The method of preparing the nerve fibres involved has been described in detail in the two above-mentioned papers. For stimulation, induction shocks were used (faradic or break shocks of variable frequency up to about 80 per second). The contractions were recorded isotonically on smoked paper or isometrically on photographic paper. In the latter case the action currents were recorded on the same paper, using a Matthews oscillograph.

Results.—We investigated the effect of stimulating the inhibitory fibre with different frequencies, the motor fibre being stimulated at a constant frequency of about 50 per second. The frequencies at which inhibition was just noticeable (minimum) and those at which it was complete (maximum) were determined. This experiment was performed on the abductor, on the

extensor of the propodite, and on both the "fast" and the "slow" contraction of the flexor of the propodite. For the contractions of the first two muscles and for the slow contraction of the third the minimum frequency was about 10 per second, with small variations partly due to fatigue; the maximum was in each case reached at about 30 per second. In contrast with this the fast contraction of the flexor had a minimum frequency of about 25 per second, whereas inhibition was not yet complete with frequen-

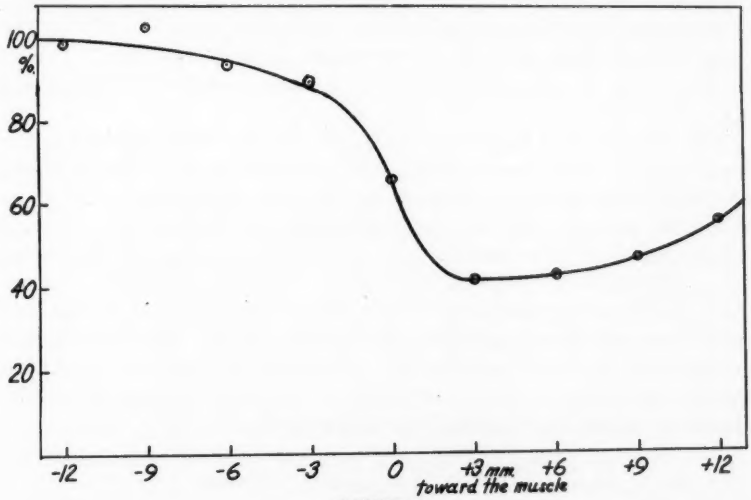

FIGURE 1

Figure showing the relation between the height of the action currents during inhibition and the position of the electrodes on the inhibiting fibre relative to those on the excitatory fibre. The abscissae give the position of the inhibitory electrodes; the ordinates represent the height of the action currents after 3 seconds of inhibition (and a total of 6 seconds of excitation) in percentage of that one second after the end of inhibition (total excitation 7 seconds).

cies of 50 per second. For this reason contractions of the fast type result when the whole nerve bundle is stimulated.

It was investigated whether the time of arrival at the muscle of the excitatory impulses in relation to that of the inhibitory ones had an influence on the contraction. For this experiment the abductor of the dactylopodite was used, and the stimulation of the two fibres was faradic. A single primary coil fed two secondaries each of which was connected by micromanipulated electrodes to one of the fibres. The moment of arrival at the muscle was changed by physiological means: large specimens were used; the exposed part of the nerve fibres was often up to 15 mm. in length; and, since the conduction velocity is about 8 m./sec., it was possible to produce

significant differences in the moments of arrival by changing the position of the electrodes up and down the two nerve fibres. Whereas the mechanical record (isotonic and isometric) show no noticeable differences and the inhibition was complete for any position of the electrodes, the size of the action currents varied from being quite unaffected up to a reduction of more than 50%. The figure shows the relation of the size of the action currents to the position of the electrodes. We cannot discuss this very interesting result here fully, but want to point out that this is evidence of the existence of two separate inhibition mechanisms, one which works directly on the contractile elements and another influencing the action currents. It is at the same time in support of the view that action current and contraction are separate phenomena.

This latter point is further illustrated by another result obtained in the same series of experiments. The action currents of the abductor show, like those of the slow contraction of the abductor, an increase in size up to a certain maximum with continued stimulation. When the impulses are so timed as not to give a reduction of the action currents during inhibition, this growth goes on though the mechanical contraction is inhibited; when the action currents undergo a reduction, they as well as the mechanical contraction attain almost immediately the size which they would have had if no inhibition had been intercalated. From this we draw the conclusion that the facilitation processes in the muscle, caused by the repetition of the excitatory stimuli, go on notwithstanding inhibition.

[1] C. A. G. Wiersma and A. van Harreveld, *Proc. Nat. Acad. Sci.*, **22**, 190 (1936).

[2] A. van Harreveld and C. A. G. Wiersma, being published.

PHOTOSYNTHESIS IN RELATION TO LIGHT AND CARBON DIOXIDE

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Communicated June 25, 1936

Several new proposals have recently been made for the specific chemical reactions concerned in photosynthesis,[1,2,3] as well as for the kinetics of the process in its relation to light and carbon dioxide.[4,5,6] In order to evaluate the various suggestions, it is necessary to have definitive measurements of these relations covering a range sufficient to render them critical. The existing data do not cover the necessary range. Moreover, it still remains to be demonstrated that measurements with one plant show fundamentally the same properties as with another. We have therefore made extensive measurements with one plant, and have compared them

with the data for other plants under conditions which show their basic similarities and differences.

The fresh water plant, Cabomba, was used throughout. Small branches suspended in buffer solution gave reproducible values even when the same piece of tissue was used on successive days. Carbon dioxide was supplied

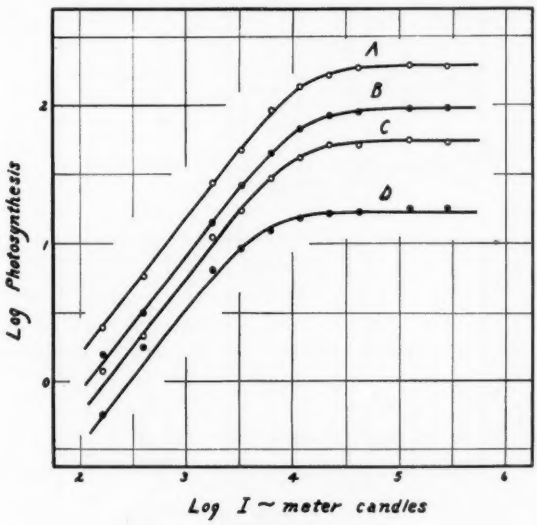

FIGURE 1

Photosynthesis as a function of light intensity for Cabomba. Photosynthesis is in terms of cu. mm. of oxygen per hour per 100 milligrams wet weight of tissue. Each curve is the average of five similar runs. The photosynthesis scale is correct only for curve A. The others have been shifted downward in order to keep the curves distinct: B by 0.2, C by 0.4 and D by 0.4 of a log unit. The carbon dioxide concentrations in moles per liter were: A, 2.90×10^{-4}; B, 1.31×10^{-4}; C, 7.87×10^{-5}; D, 2.05×10^{-5}. White light was used and the temperature was 25.3°C. The same curve is drawn through all of the data and is from equation (1).

from Warburg's carbonate mixtures using the potassium salts. Potassium bicarbonate was used to obtain the highest carbon dioxide concentration which was estimated from its pH as determined with a glass electrode. In making the measurements the buffer solution was changed as often as necessary to prevent an effective decrease in CO_2 concentration. Light was furnished by a 500-watt projection lamp in conjunction with a mirror and optical system so arranged as to give at the bottom of the manometer

vessel a maximum illumination of nearly 300,000 meter candles (Lux). The intensity was varied with neutral filters made from photographic plates which were calibrated *in situ* with a Macbeth illuminometer. The high illumination enabled us to cover an intensity range of 1 to 1700, the maximum previous range having been approximately 1 to 200. Photo-

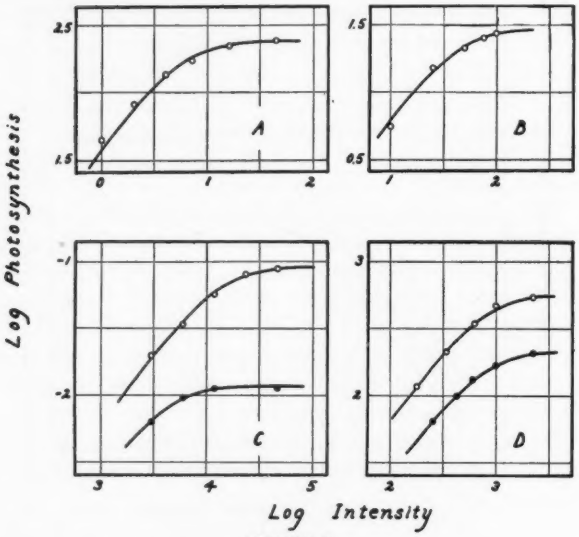

FIGURE 2

Photosynthesis as a function of light intensity, the data of various investigators: *A*—Warburg on Chlorella; *B*—Emerson and Green on Gigartina; *C*—Willstätter and Stoll on Ulmus yellow leaves (open circles), and on Ampelopsis (solid circles); *D*—Van der Paauw on two varieties of Hormidium, Pringsheim's strain (open circles) and Van den Honert's strain (solid circles). The data are given in the original units of the various authors. The curve drawn through the data is from equation (1).

synthesis was measured as oxygen production with the Warburg manometric technique.[7]

Figure 1 presents the rate of photosynthesis as a function of light intensity for four different carbon dioxide concentrations. Though each point is the average of five separate experiments, the data of the individual experiments are uniform with the averages, except for the two lowest intensities where the photosynthesis is near the compensation point.

The curve drawn through the data has the equation

$$KI = p/(p^2_{max} - p^2)^{1/2} \qquad (1)$$

where p is the rate of photosynthesis at light intensity I, K is a constant which locates the curve on the I axis, and p_{max} is the asymptotic maximum rate of photosynthesis. When log p is plotted against log I, the shape of the curve is independent of the constants K and p_{max}, a fact which facilitates comparison with the data. Curves similar to that in figure 1 but

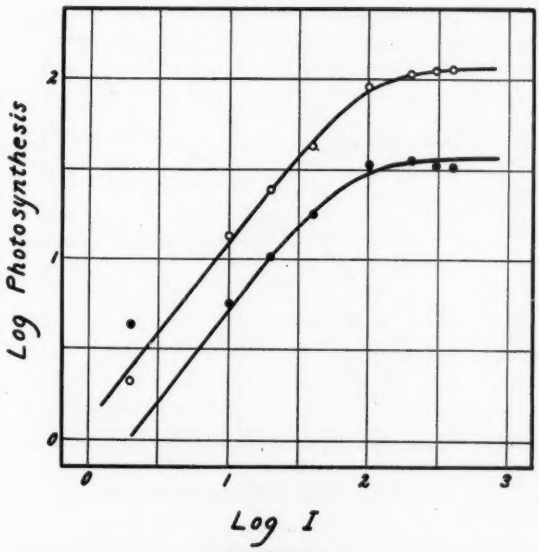

FIGURE 3

Emerson's data on two strains of Chlorella, one of high (open circles) and the other of low chlorophyll concentration. The lowest points represent readings below the compensation point and are of low precision. The same curve has been drawn through both sets of data and is from equation (1).

differing in slope and inflection result from changing the exponents in equation (1). For example,

$$KI = p/(p_{max} - p)^{1/2} \qquad (2)$$

yields a curve which is almost the same as in figure 1 but differs slightly in the rate at which it becomes parallel to the log I axis at high illuminations. The three upper sets of data in figure 1 fit equation (1) with better precision than (2), while the lowest set of data fit (2) somewhat better. Since no certain choice is at present possible, the curve for equation (1) has been drawn in all four series. Exponents other than those in (1) and (2) are definitely excluded, as for example, in equation

$$KI = p/(p_{max} - p) \quad (3)$$

used by other observers.

Figures 2 and 3 give some of the data of previous workers.[7,8,9,10,11] The curve for equation (1) has been drawn through them all. In view of the variety of plants, of experimental conditions, and of method, it is remark-

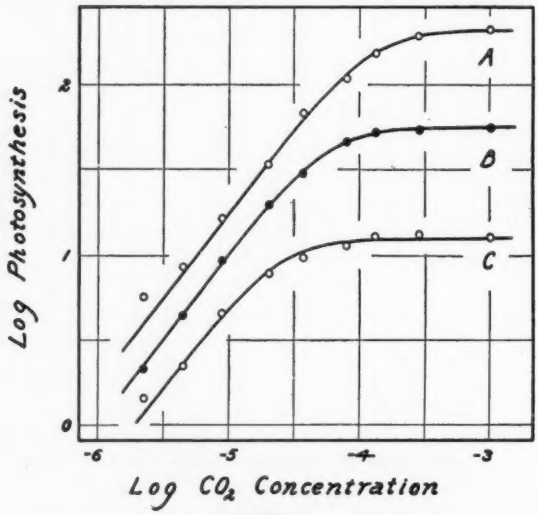

FIGURE 4

Measurements on Cabomba with different carbon dioxide concentrations at constant light intensity. Photosynthesis in cu. mm. of oxygen per hour per 100 mg. wet weight of tissue. The scale is correct for curve *A*; curve *B* has been moved down 0.4 and curve *C*, 0.6 of a log unit. The temperature was 25.3°C. and red light beyond 580 mμ obtained with No. 246 Corning filter was used. The relative intensities were: *A*, 282,000; *B*, 27,200; and *C*, 6300, which are the intensities in meter candles of the unfiltered light. Each curve represents the averages of five similar runs. As in the preceding figures, the curve from equation (1) has been drawn through the data.

able that the data fit so well an equation as specific as the one drawn through them. While some of the data presented (e.g., those of Warburg) fit equation (2) a little better, none of them are adequately represented by equation (3). Of the measurements not shown in figures 2 and 3, those of Van den Honert[12] are identical with Van der Paauw's on the same material. The experiments of Harder on Fontinalis[13] have too high an experimental error to be critical. The data of Hoover, Johnston and

Brackett[14] do not go to high intensities but are consistent with the others shown. The remaining data in the literature are too sparse and inaccurate for critical use.

Figure 4 presents the rate of photosynthesis as a function of carbon dioxide concentration for several different illuminations with Cabomba. The curve drawn through the data is the one used in figures 1, 2 and 3, and is from equation (1) with carbon dioxide concentration substituted for light intensity. Apparently the rate of photosynthesis varies in the same way with both light intensity and CO_2 concentration.

Comparison of previous results among themselves and with ours is

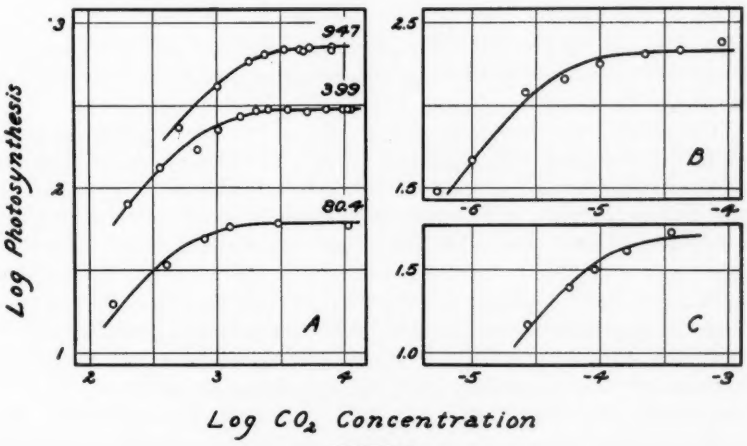

FIGURE 5

A. The data of Hoover, Johnston and Brackett on young wheat. The numbers on the curves give the light intensity in foot-candles. *B*. Warburg's data on Chlorella. *C*. Those of Emerson and Green on Gigartina. The same curve as in the preceding figures has been drawn through these data.

difficult because the method of supplying carbon dioxide influences the results. Warburg, and Emerson and Green, supplied carbon dioxide from buffer mixtures similar to those used here; their data can therefore be compared directly with ours. This is done in figure 5 *B*, *C*. The agreement with equation (2) is not so good as desired; this may be because the data represent only single experiments. In Emerson and Green's work on Gigartina this is further complicated by the use of a different piece of tissue for each point. Figure 5 *A* gives the data of Hoover, Johnston and Brackett for three light intensities obtained with young wheat. Carbon dioxide was supplied in gas mixtures circulated rapidly through an enclosed chamber. Their data are easily fitted with equation

(1). However, the data of Van den Honert and Van der Paauw on Hormidium using gas mixtures do not resemble the other measurements. This may be due to the fact pointed out by Emerson[15] that external diffusion rate is probably the limiting factor in these experiments since at low carbon dioxide tensions Q_{10} is unity, whereas in the experiments of Warburg and of Emerson with Chlorella with buffer mixtures Q_{10} is high.

The fact that photosynthetic rate is the same function of both carbon dioxide concentration and light intensity argues for simplicity of the kinetic mechanism. Still, the presence of a fractional exponent indicates a complex system. Squaring both sides of equations (1) and (2) eliminates the square root, but intensity and CO_2 concentration now enter as the square. This may indicate a chain process with perhaps more than one light reaction, and would be in keeping with Warburg and Negelein's discovery[16] that four quanta are necessary for the reduction of a single CO_2 molecule.

The kinetic equations of Arnold, of Baly and of Burk and Lineweaver, all reduce to equations identical in form with (3), which we know does not fit the measurements. Arnold has not attempted to fit his equations to the data of intensity and carbon dioxide concentration, while Baly used only the intensity data of Warburg and did not obtain a satisfactory agreement with them. Burk and Lineweaver used Harder's data, which have so high an experimental error that they are not critical. The fact that the data here presented, both original and from others, do not fit the equations derived by the above investigators provides a specific criticism of their equations. Our own equations (1) and (2) are to be considered as purely empirical descriptions of the data; actually they are variants of the general photostationary state equation used by Hecht[17] for the visual process, and may represent basically the same ideas. However, no matter what their derivations are, the equations primarily describe the measurements, and can be used as a criterion for the validity of any theoretical description of photosynthesis.

A more complete discussion and the details of this work are expected to be published in the *Journal of General Physiology*.

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ON THE PHYSIOLOGY OF THE FORMATION OF NODULES ON LEGUME ROOTS

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Communicated June 18, 1936

It is now about 50 years since Hellriegel and Wilfarth finally elucidated the nature and importance of the nodules on the roots of *Leguminosae*, and Beijerinck obtained the nodule bacteria in pure culture. Nevertheless on the three essential points in regard to the nodules we are just as much in the dark as ever. These are:

1. How does the nodule fix nitrogen? The bacteria alone, in culture, fix no nitrogen, neither does the plant without them.

2. How do the bacteria enter the root? They pass through the cell-walls with apparent ease, yet in culture they do not attack cellulose.

3. How does the nodule develop? Morphologically, of course, its development has been described many times, but how do the bacteria bring about the proliferation and enlargement which constitute the nodule?

The present note is an attempt to answer the third of these questions, and to put forward a physiological theory of nodule formation, which is strongly supported, if not proved, by the facts.

Studies upon the plant growth hormones, or auxins, especially in relation to roots, have established the following facts (for literature up to Dec., 1934, see the author's review[1]):

a. A large number of substances, most, if not all, of which are unsaturated organic acids of various types, or their esters, have plant-growth-promoting activity. Not only the actual activity, by assay depending on cell-elongation, but also the ability of the substances to be transported in plant tissues, varies widely.[2] Indole-3-acetic acid is one of the most active.[3]

b These auxins act not only on cell elongation, but they also promote, and are necessary for, the formation of roots.[1]

c. They also inhibit the elongation of roots very strongly.[1,4]

d. In the pea at least they promote the formation of branch or lateral roots on the main root.[5]

e. They stimulate cambial activity in the stem.[6]

f. Under certain conditions they may cause swelling or enlargement of the root tissues, principally of the cortex.[7]

g. Indole-3-acetic acid, and possibly other active substances, are frequently produced by bacteria and fungi in culture.[1]

On the other hand, studies on the morphology of the nodules have shown that they arise either in the cortex or, like lateral roots, in the pericycle (for literature see[8]). They consist of greatly enlarged cells containing the bacteria, while outside the infected cells other cells are stimulated to growth and division. There is therefore strong indication that a substance causing cell enlargement and division is produced in, and diffuses out of, the bacterially infected cells.

Combining these two groups of facts we get the following picture of the series of events after the bacteria enter the root tissues; the bacteria multiply in the cells, causing breakdown of carbohydrate and protein. In their metabolism is produced, among other things, small amounts of an auxin. This substance causes enlargement of the cells in which it is produced, and also, being readily diffusible, enters the pericycle behind the cortical tissue inhabited by the bacteria, and there also stimulates growth and division, giving rise to the first stages of a lateral root initial (*d* above). However, since auxin production continues, this lateral root is prevented from elongating (*c* above). Instead its cells increase in size isodiametrically (*f* above), while certain of the uninfected cells are stimulated to division (*e* above) by auxin diffusing out of the infected area. In this way we get a shapeless mass of parenchymatous tissue, which is essentially a lateral root prevented from elongating. The differentiation of the vascular system within the nodule offers a complication which cannot be dealt with here, as nothing is yet known about the causes of differentiation.

Such a picture is in line with all the known facts. Can it be supported experimentally?

In the first place, if auxin is produced in the nodule, it must be possible to detect it by the standard Avena technique. Direct diffusion experiments were therefore made. Young, still growing nodules from roots of *Pisum* plants 3–4 weeks old were sectioned and placed on plain agar. The agar was then tested upon Avena coleoptiles, and its auxin activity was considerable. This is in marked contrast to root-tips which do not, in general, yield auxin to plain agar, but only to agar containing dextrose or other nutrient. The nodule appears to possess no marked polarity since about the same number of units diffuses into the agar when either its apical or its basal half is used, the area of contact being a cut through the

center parallel to the root in each case. The auxin is therefore not due to the meristematic tip of the nodule, but comes directly from the infected tissue. The maximum yield, 10–12 plant units per nodule in 3 hours' diffusion, was attained with nodules 2–3 mm. in diameter. As soon as the nodule turns brown and soft, auxin production ceases completely. Lateral root-tips similarly treated gave up only traces of auxin to the agar. The nodule is therefore an active auxin-producing center, its auxin production roughly paralleling its growth.

In the second place, the application of auxin, locally, to very young lateral roots (0.5 mm. long) of the same plant resulted in their complete inhibition. The root-tip remained more or less conical, but after 4 days there was swelling at its base. There was no generalized swelling of the main root. In one case two lateral root initials grew together to produce a nodule-like object. The swelling was due to increase in size of the cells of the cortex. It is hardly to be expected that application of the active substance from outside would imitate its action when applied from within by the bacteria, but at least, the complete inhibition of elongation of the lateral rootlet is confirmed.

Lastly we come to the nature and source of the auxin produced by the bacteria. The auxin produced by *Rhizopus* is indole-acetic acid formed from the tryptophane present in the peptone of the culture medium.[1] The ability of bacteria to carry out this reaction is fairly general, especially, according to Frieber,[9] if they do not produce free indole from the tryptophane. This the *Rhizobium* bacteria do not do.[10] Further, it has been observed by the author many times that the application of indole-acetic acid solution to roots results not only in inhibition, but also in a swelling which is characteristically due to *radial elongation* of the cortical cells, together with divisions in the cambium and pericycle. These two characteristic features are exactly what was shown to be produced by sterile filtrates of nodule-bacteria cultures (Molliard[11]). He found marked inhibition of elongation of the root, division in the pericycle and radial elongation in the cortex. There is no doubt, therefore, that the bacteria in culture do produce auxin in considerable amounts.

Experiments were also carried out to determine whether uninfected parts of the pea plant could convert tryptophane to indole-3-acetic acid; no indications of the ability could be found, hence the presence of the bacteria is essential. The amount would be small, being presumably limited by the cell protein, but the effects are in any case of the chronic rather than the acute type.

Taken together, the above facts, new and old, thus provide a satisfactory explanation for the growth of the nodule and the rôle of the bacteria in its formation.

Similar considerations, with some modifications, would apply to galls

and other outgrowths of the plant. The analysis of these phenomena will be dealt with at a later date. Detailed experimental data on the nodules will be published elsewhere.

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THE NATURE OF TONAL BRIGHTNESS

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It is well known that tones can be characterized as *bright* or *dull*. When intensity does not alter greatly, tonal brightness seems to vary with pitch: high tones are bright, and low tones are dull. Thus Rich[1] concluded that brightness and pitch are identical as tonal attributes. Abraham[2] concluded that the brightness of tones produced by a Seebeck siren is a function of the ratio of the size of the hole in the siren disc (H) to the size of the closed interval between holes (I). For values of H/I from about 1.0 to 0.1 he concluded that brightness varies inversely with H/I. By an analysis of what he supposed these waves forms to be he decided that brightness is not due to the presence of higher partials, for the reason that his analyses did not persistently show an increase in the higher partials for the brighter tonal complexes. He thus was led to suppose that brightness depends upon the form of the primary wave in the complex. Ogden[3] later accepted Abraham's conclusion.

Apparatus.—For stimuli we cut sirens from cardboard (0.04 in. thick). Each siren is a disc of 16-in. diam. The holes are sectors, 0.75 in. in radial dimension, with the ends cut exactly along radii and the sides cut approximately as arcs about the siren's center. In this way we avoided the ambiguity that arises with the circular holes of the Seebeck siren, where it is hard to say just what is the exact duration of the hole and of the interval be-

tween holes. Moreover, the radial width of these holes is so large in relation to the spread of the blast of air that it probably constrains the air very little.

The air-blast was delivered from a tank at a pressure of 5 lb. per sq. in. The nozzle pressure was reduced by the friction of a long rubber tube (16 ft. long, $^1/_4$ in. inside diam.). The nozzle was a piece of $^1/_2$-in. round brass, drilled with a hole 0.16 in. diam., stream-lined conically back $^1/_2$ in. from the end. The orifice was fixed about 0.25 in. from the face of the disc.

The sirens were rotated on induction motors, which ran as synchronous motors at 1800 r. p. m. on light loads, and which maintained great constancy at 1650 r. p. m. with the sirens as loads.

Among 24 stimuli prepared for investigation, the nine listed in table 1 yielded the most significant results. The stimuli 1*B*, 2*B* and 3*B* are identical. 1*A*, 1*B* and 1*C* provide variation of the mean linear velocity (*V*) of the holes, with *H*/*I* and the frequency (*F*) constant. 2*A*, 2*B* and 2*C* give variation of frequency (*F*), with *H*/*I* and *V* constant. 3*A*, 3*B* and 3*C* provide variation of the ratio of the hole to the interval between holes (*H*/*I*), with *V* and *F* constant. Preliminary experimentation with other discs in which *H* varied with *I* constant, and *I* varied with *H* constant, revealed no significant auditory changes other than those dependent on the ratio *H*/*I*.

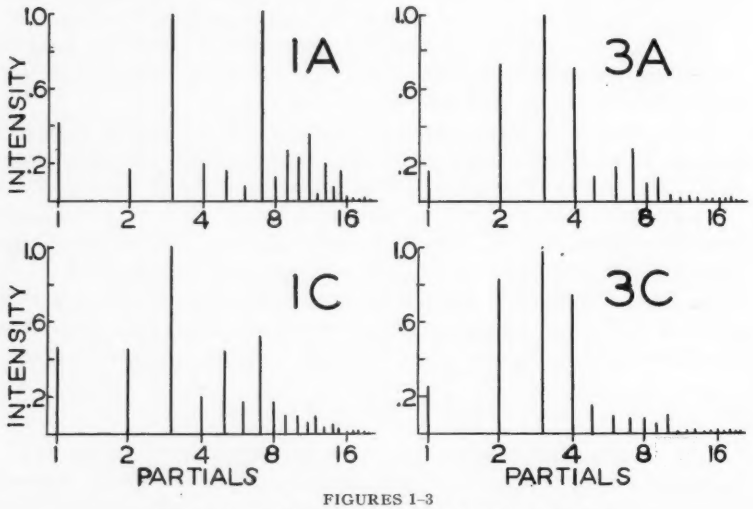
TABLE 1

DESCRIPTION OF STIMULI

STIMULUS	NO. HOLES PER CIRCUM. *N*	MEAN RADIUS OF HOLES (IN.) *R*	SIZE OF HOLE (DEG.) *H*	SIZE OF INTERVAL (DEG.) *I*	LINEAR VELOCITY (CM./SEC.) *V*	FREQUENCY (C. P. S.) *F*	RATIO *H*/*I*
1*A*	12	6.0	15	15	264	330	1.0
1*B*	12	4.5	15	15	198	330	1.0
1*C*	12	3.0	15	15	132	330	1.0
2*A*	18	4.5	10	10	198	495	1.0
2*B*	12	4.5	15	15	198	330	1.0
2*C*	9	4.5	20	20	198	247	1.0
3*A*	12	4.5	5	25	198	330	0.2
3*B*	12	4.5	15	15	198	330	1.0
3*C*	12	4.5	25	5	198	330	5.0

Velocity.—These sirens yield complex tonal masses and a simple comparative judgment of two such complexes is not easy. However, there is no exception (among 5 observers) to the rule that brightness varies directly with linear velocity (*V*). (See table 2.) So also does loudness. All observations show that 1*A* is brighter and louder than 1*B*, and 1*B* brighter and louder than 1*C*. When the loudness of the two members of the comparison are equated subjectively by throttling the tube to the nozzle for the louder sound, the difference in brightness is much reduced, the judgment of brightness becomes more difficult, and two of the five observers reversed their judgments for one or the other of the two stimulus pairs. It is apparent

that brightness is not loudness, since a difference in brightness persists when the difference in loudness is eliminated, but it also appears that brightness tends to vary directly with loudness. Since it is known that brightness also tends to vary with pitch,[1] we advance the hypothesis that brightness varies directly with both the intensity and the frequency of the stimulus, after the manner of tonal density.[4,5]



FIGURES 1–3

Harmonic spectra of the sounds $1A$, $1C$, $3A$ and $3C$.

The paired stimuli ($1A$–$1C$ and $3A$–$3C$) are subjectively equated in loudness. Abscissa: logarithmic scale of frequencies of harmonic partials. Ordinate: intensity as ratio of third partial = 1.0. The properties of the stimuli are:

Stimulus:	$1A$	$1C$	$3A$	$3C$
Velocity (cm./sec.) = V	264	132	198	198
Ratio = H/I	1.0	1.0	0.2	5.0
Frequency (c.p.s.) = F	330	330	330	330

Figure 1 shows that brightness depends on V, since $1A$ is brighter than $1C$ when H/I and F are constant.

Figure 3 shows that brightness does not depend on H/I, since $3A$ and $3C$ are not demonstratively different in brightness when H/I varies, and V and F are held constant.

The frequency spectra for $1A$ and $1C$ are shown in figure 1. These analyses of the sounds were made by means of a General Radio Type 636-A Wave Analyzer. They gave no evidence of Abraham's *Knall* frequency, well below the siren's fundamental, or of any other frequencies below the fundamental. A few inharmonic partials were found, but they were of insufficient intensity to appear in the charted spectra. Figures $1A$ and $1C$

show the harmonic components of the stimuli 1*A* and 1*C*, plotted as ratios of the third partial as unity, for all these tonal complexes that have been analyzed turn out to be dominated by the third partial. Since the analyzed stimuli were subjectively equated for loudness, the charts must be examined for the effect of frequency upon brightness. It will be seen that the brighter sound, 1*A*, weights the upper partials more than does the duller sound, 1*C*. 1*A* has less of the first and second partials than 1*C*, has the seventh partial fully as strong as the third, and has the partials in the fourth octave (8 to 15) more prominent than they are in 1*C*. The visually perceived difference between the plotted spectra is quite as great as the auditorially perceived difference between the brightness of the two sounds, for

TABLE 2

COMPARATIVE JUDGMENTS FOR DIFFERENT PAIRS OF STIMULI

At the left is shown the category of judgment (and whether loudness was or was not equalized). Each column shows the two frequencies with which each of the two stimuli was preferred as brighter, etc. The table summarizes 136 judgments. Among these there were only 8 equality-judgments, and these have been divided equally between the two sides of the column. Thus "7–0" in the upper left corner of the table means that the sound 1*A* (V = 264 cm./sec.) was 7 times judged brighter than the sound 1*B* (V = 198 cm./sec.), and no times less bright.

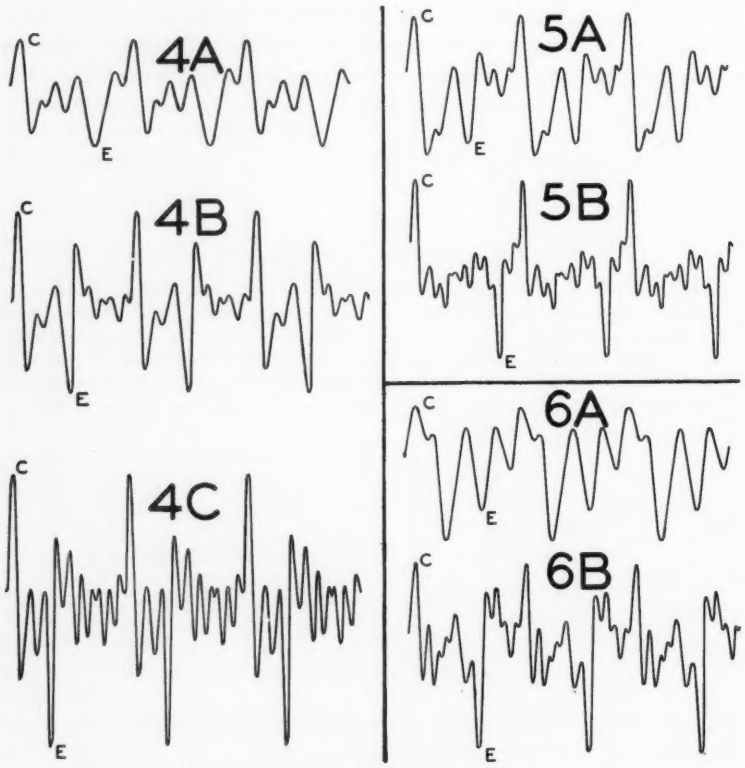
	VELOCITY (V) EQUALS				FREQUENCY (F) EQUALS				*H/I* EQUALS			
	264 1*A*	198 1*B*	198 1*B*	132 1*C*	495 2*A*	330 2*B*	330 2*B*	247 2*C*	0.2 3*A*	1.0 3*B*	1.0 3*B*	5.0 3*C*
Brighter (loudness not equal)	7	0	7	0	7	0	6.5	0.5	4.5	2.5	2	5
Louder	7	0	7	0	5.5	1.5	3.5	3.5	6.5	0.5	0	7
Brighter (loudness equal)	5	1	6	0	4	2	5	1	3.5	2.5	2	4
Denser (loudness not equal)	4	0	4	0	2	2	4	0	...	...	.	.

these spectra are taken for the small difference of brightness that occurs when the sounds are of equal loudness.

Figures 4–6 also demonstrate the dependence of brightness upon velocity and its independence of the ratio H/I when frequency is constant. These graphs are wave-forms traced from the patterns on a cathode-ray oscillograph. The sounds were got from a cardboard Seebeck siren with round holes. The properties of their stimuli are shown in the legend of the figures. 4*A*, 5*A* and 6*A* are the dull sounds; 4*C*, 5*B* and 6*B* are brighter (and also louder). 4*B* lies qualitatively between 4*A* and 4*C*. The series 4*A*–4*B*–4*C* duplicates Abraham's condition and shows brightness varying directly with V and inversely with H/I. The pair 5*A*-5*B* shows brightness varying directly with both V and H/I. The pair 6*A*-6*B* shows brightness varying directly with V when H/I is constant. The conclusion is inescapable that

it is a change in the velocity V, and not the change in the ratio H/I, which causes the difference in brightness.

Figures 4–6 also show the typical appearance of the wave-forms of bright sounds ($4C$, $5B$, $6B$) and of dull sounds ($4A$, $5A$, $6A$).



FIGURES 4–6

Oscillograms of seven siren sounds.

The sounds increase in brightness in the orders $4A$–$4B$–$4C$, $5A$–$5B$ and $6A$–$6B$. They are not equated for loudness. C = the compression-phase when the hole opens, and E = the expansion-phase when the hole closes. The properties of the stimuli are:

Stimulus:		$4A$	$4B$	$4C$	$5A$	$5B$	$6A$	$6B$
Velocity (cm./sec.)	$= V$	250	750	1040	485	955	475	1085
Ratio	$= H/I$	1.30	0.65	0.35	0.75	3.60	1.0	1.0
Frequency (c.p.s.)	$= F$	165	165	165	132	132	132	132

Figure 4 shows that brightness increases when V increases and H/I decreases.

Figure 5 shows that brightness increases when V increases and H/I increases.

Figure 6 shows that brightness increases when V increases and H/I is constant. Hence brightness depends upon V and not upon H/I.

Frequency.—The series of stimuli $2A$-$2B$-$2C$ gives a decrease of frequency (F) when V and H/I are constant. In all cases $2A$ is judged brighter than $2C$, and usually $2A$ is also judged to be louder, although the difference in loudness seems less than the difference in brightness. $2B$ was judged brighter than $2C$ in all cases but one. They are nearly equal in loudness, and there is no consistency as to the direction in which the difference of loudness is reported. When the differences of loudness are eliminated by subjective equation, the order of decreasing brightness is still $2A$-$2B$-$2C$, although here two observers showed confusion in distinguishing between $2A$ and $2B$.

This finding supports the preceding conclusion that brightness tends to increase when the frequencies of the various components of a tonal complex are increased, or when the total intensity of the complex is increased.

The H/I Ratio.—The series $3A$-$3B$-$3C$ provides an increase in H/I with V and F constant. This is the parameter which Abraham accepted as the condition of brightness. We do not find it possible to obtain consistent comparative results for brightness with these stimuli. It is clear that the stimulus $3B$ is not so loud as $3A$ and $3C$. Hence there is a tendency for observers to report $3A$ and $3C$ as brighter than $3B$. When the loudnesses of the pairs are equated subjectively, the judgments of brightness become difficult and contradictory. See table 2. The harmonic spectra of $3A$ and $3C$, shown in figure 3, support this conclusion: the spectra are not obviously different. It is now clear that brightness does not depend upon the H/I ratio, except that the departure of this ratio in either direction from unity tends to increase loudness, and with it brightness in lesser degree.

A similar conclusion has already been reached from a consideration of the wave-forms of figures 4–6.

The Relation to Abraham's Finding.—The chief ground for Abraham's conclusion is found in his data for the relative brightness of sounds obtained from a special Seebeck siren. In this siren, on each of five different circumferences, 24 circular holes were cut. All holes were the same size. On the circumference of least radius the diameter of a hole approximately equaled the space between the holes, i.e., $H = I$, and $H/I = 1.0$. On the outer circumference, since the holes were not made larger, the space between holes became about ten times as large as the holes; $H/I = 0.1$. Abraham found that the brightness of the sound increased consistently as H/I decreased from 1.0 to 0.1. However, he had not controlled the velocity, which was more than five times as great on the outer circumference as on the inner. The changes of brightness which he observed should have been ascribed to V and not to H/I.

Abraham considered the possibility that brightness depends upon the predominance of upper partials and rejected the hypothesis. He worked without the advantage of modern means for the electrical analysis of sounds

and he assumed incorrectly that a siren, in which $H/I = 1.0$, would have a simple wave-form with the phases of compression and expansion unequal. Figure 4 shows what actually happens to the wave-form when H/I varies under Abraham's conditions with V, and figure 3 shows what happens to the harmonic analysis when H/I varies without V. The general conclusion, based upon the inspection of oscillograms, is that the brighter siren sounds tend to have more and sharper peaks—a conclusion that is consistent with the finding that they have relatively strong upper partials.

The Relation of Brightness to Density.—One of us (Stevens) has shown that tones possess an attribute of density, which increases with the intensity or the frequency of the stimulus, and can be held constant if intensity and frequency be varied inversely in the right amounts.[4,5] We have shown that brightness is a similar function of intensity and frequency. The question arises as to whether brightness and density can be identified as the same tonal attribute.

Neither of us is able to distinguish between brightness and density in making these comparative judgments. One of us (Stevens) is accustomed to making judgments of tonal density and asserts that at no time has he ever been able to distinguish density from brightness. The other of us (Boring) learned to judge density after he had learned to judge brightness, and, although his mechanisms of judgment (visual surrogates) are different for the two tasks, he finds that brightness and density always covary and are thus indistinguishable for him as attributes of the sound. Two other observers have given judgments of density (see table 2) and in most instances have reported the relations of stimuli with respect to density the same as they had previously reported for brightness. One of these observers had been trained to make judgments of density in a previous experiment.[4] However, these observers both reported $2B$ (330 c.p.s.) as denser than $2A$ (495 c.p.s.) but $2A$ as brighter than $2B$. After repeated trials neither of us is able to agree with this inversion. We think that the other observers have shifted their criteria in these cases and are judging the coherence of the tonal mass and not the attribute that has been established as density. These tonal masses offer in the patterns of their complications many new dimensions of difference, and the maintenance of a single criterion is doubtless more difficult than it is for pure tones.

Working with pure tones, one of us (Stevens) has tried to get observers to make two tones of different frequencies equal in brightness by varying the intensity of one tone. This is the method that has been used to establish the laws of variation for the other tonal attributes—pitch, loudness, volume and density. Observers, who are accustomed to making judgments of density, find it impossible to equate these two tones in brightness, if they are instructed to regard brightness as different from density. On the other hand, observers, who are unfamiliar with density, are able to make

consistent judgments of brightness, and for them brightness turns out to increase with both the intensity and the frequency of the stimulus, according to the relationship which has with other observers been established for density.

It seems to us, therefore, that brightness and density covary to such an extent that the two attributes ought, at least tentatively, to be identified. It is probable that two attributes, whose laws of dependence upon the parameters of the stimulus differ by an amount that is small with respect to the probable error of the judgments, would never come to be distinguished. They would remain practically identical until the probable error of judgment could be reduced. In this sense at least we think that tonal brightness is tonal density as that attribute has been previously established.[4]

Conclusion.—The brightness of tones and tonal complexes varies directly with the intensity and with the frequency of the stimulus, after the manner of tonal density. Tonal brightness and tonal density are thus probably to be identified. Of the two terms *density* is to be preferred, since it has been definitely established by quantitative measurement and is therefore less equivocal than *brightness*.

[1] G. J. Rich, "A Study of Tonal Attributes," *Amer. Jour. Psychol.*, **30**, 121–164 esp. 153–158 (1919).

[2] O. Abraham, "Zur psycholgischen Akustik von Wellenlänge und Schwingungszahl," *Z. f. Sinnesphysiol.*, **51**, 121–152, esp. 130–152 (1920).

[3] R. M. Ogden, *Hearing*, New York, Harcourt Brace and Co., esp. 10–12, 58–62 (1924).

[4] S. S. Stevens, "Tonal Density," *Jour. Exper. Psychol.*, **17**, 585–592 (1934).

[5] S. S. Stevens, "The Attributes of Tones," *Proc. Nat. Acad. Sci.*, **20**, 457–459 (1934).

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*THE ACTION OF INTERMEDIN ON CRUSTACEAN MELANOPHORES AND OF THE CRUSTACEAN HORMONE ON ELASMOBRANCH MELANOPHORES**

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In two previous papers physiological and chemical evidence indicating a close similarity between the chromatophorotropic hormone of the crustacean eye-stalk and that of the vertebrate pituitary gland has been presented (Abramowitz[1,2]). It was found that removal of the eye-stalks of Portunid crabs resulted in the concentration of the melanophore pigment, a response identical with that following the removal of the hypophysis in elasmobranchs, amphibians and some reptiles. It was shown furthermore

that a purified extract of the eye-stalks of *Palaemonetes* could restore the dark coloration of a completely hypophysectomized teleost, amphibian or reptile. The solubility affinities of the active crustacean material were also found to be similar to those of intermedin[3] in many respects.

The appearance of the dogfish, *Mustelus*, at Woods Hole during the month of May afforded an opportunity to test further the action of the crustacean hormone on vertebrate chromatophores. The newly-born dogfish pup served as a convenient laboratory animal. Most of the pups were obtained from the uteri of pregnant females. After immersion for two minutes in a mixture of cracked ice and sea water, the pups were hypophysectomized by a direct oral route. Following the removal of the hypophysis, the animals assume a pink hue within four hours. This pink hue is due mainly to extreme melanophore pigment concentration and to some extent to cutaneous vasodilation. Hypophysectomized pups retain this hue indefinitely regardless of background influences or of light intensity. Extracts of the eye-stalks of two crustaceans, *Libinia* and *Callinectes*, were tested. The eye-stalks were macerated in sea water, the supernatant fluid boiled and filtered, and the filtrate concentrated by evaporation so that 1 cc. of filtrate contained the extract of 20 eye-stalks. Injection of 1 cc. of this crude extract, either from *Libinia* or from *Callinectes*, intramuscularly into normal black-adapted pups produced no pigmentary changes. Injections of the same extract in the same dosage into hypophysectomized pups was followed by melanophore expansion, a reaction which persisted for 5 hours. Thus the crustacean hormone is effective in dispersing the pigment of an hypophysectomized elasmobranch. The dispersion of melanophore pigment following treatment with crustacean eye-stalk hormone has now been shown to occur in a member of each of 4 groups of vertebrates.

While the invertebrate hormone is capable of affecting the chromatophores of vertebrates, the reciprocal action, that of the vertebrate pigmentary hormone on the chromatophores of invertebrates, has not been demonstrated. To determine the effect of intermedin, the vertebrate pigmentary hormone of the intermediate lobe of the hypophysis, on the crustacean melanophore, the fiddler crab *Uca* was used as a test object. Megašur[4] and Carlson[5] have shown that *Uca* pales following the removal of its eye-stalks, and Carlson has added furthermore that the dark coloration may be restored by the injection of the eye-stalk hormone. The blinded fiddler crab is therefore an excellent test animal for, like the hypophysectomized frog, its melanophores remain in a state of pigment concentration despite background influences or light intensity. Crude extracts of the eye-stalks of *Uca*, prepared as described above, when injected[6] into specimens whose eye-stalks had been removed 2 days previously, elicited a maximal dispersion of the black pigment, a response which persisted for 7 hours. Injection of intermedin[7] into 40 similarly prepared specimens evoked the same

response. In both sets of experiments the animals regained their pale condition 8 hours following the time of injection, and could then be darkened again by another injection.[8]

The vertebrate pigmentary hormone is thus capable of evoking responses of invertebrate chromatophores, just as the crustacean hormone is capable of acting on the chromatophores of vertebrates as first demonstrated by Koller and Meyer[9] and Kropp and Perkins.[10] The evidence for the agreement in response to the vertebrate and invertebrate pigmentary hormones, at least as far as the reactions of the melanophores are concerned, seems, therefore, to be complete.

* Aided in part by a grant from the NATIONAL RESEARCH COUNCIL Committee on the Problems of Sex administered by F. L. Hisaw.

[1] Abramowitz, A. A., *Proc. Nat. Acad. Sci.*, **21**, 678 (1935).

[2] Abramowitz, A. A., *Proc. Soc. Exp. Biol. Med.*, **34**, 714 (1936).

[3] The term "intermedin" is used here to denote the pituitary extract which evokes melanophore and erythrophore expansion in the fish, *Phoxinus*. Some authors now regard the melanophore stimulator and the erythrophore stimulator as two distinct pituitary principles.

[4] Megašur, F., *Arch. Entwick.-mech.*, **33**, 462 (1912).

[5] Carlson, S., *Proc. Nat. Acad. Sci.*, **21**, 549 (1935).

[6] 0.05 cc. containing the extract equivalent to one-half of a single stalk was the injected dosage.

[7] The intermedin used in these experiments was prepared from whole sheep pituitary powder by Prof. H. L. Gevold. The injected dosage employed was 0.05 cc. containing the extract of 0.05 gram of sheep pituitary powder (whole gland).

[8] The pigmentary system of *Uca* contains black, red, white and yellow chromatophores. Only the reactions of the melanophores are discussed in this report.

[9] Koller, G., and Meyer, E., *Biol. Zentralbl.*, **50**, 579 (1930).

[10] Kropp, B., and Perkins, E., *Biol. Bull.*, **64**, 226 (1933).